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DEGENERATIVE CHANGES IN THE MALE GERMINAL EPITHELIUM IN ACUTE ALCOHOLISM AND THEIR POSSIBLE RELATIONSHIP TO BLASTOPHTHORIA *

CARL VERNON WELLER, M.S., M.D.

(From the Department of Pathology of the University of Michigan, Ann Arbor, Mich.)

In a brief preliminary report [1] upon a small series of cases, and in a more extended abstract based upon a larger series,[2] I have summarized evidence in support of the belief that the changes in the human testis in acute alcoholism furnish a morphological basis for assuming for ethyl alcohol a blastophthoric effect upon human germ plasm. In the present paper this evidence is presented in detail for the first time, with a survey of the literature dealing with this method of approach to the question of alcoholic blastophthoria and a discussion of its implications.

The general acceptance by practically all of the pure biologists of the doctrine of the non-inheritance of acquired characteristics has had a deterrent effect upon the growth of our knowledge of alteration of the germ plasm through extrinsic factors. This has doubtless been due to the fact that changes in the germ plasm have been thought of as manifested particularly through the transmission or non-transmission of unit characters. Between such alterations which follow the recognized laws of genetics and the imposition of altered metabolism with resulting variation in potentiality for growth and differentiation there seems, at first, to be but little in common. In the future we may come to realize that essentially the same forces are operating in each type of change, crudely and diffusely in one, precisely and delicately in the other. The difficulty has been that with the biological conception of the non-

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inheritance of acquired characters emphasis has been placed upon the *stability* of the germ plasm. Actually, the germinal epithelium, particularly in certain stages of its maturation, is one of the most labile of all cell types, and it is the *lability* of the germ plasm which should be stressed.

Under the term *blastophthoria* may be grouped all of the processes of alteration, whether detrimental or beneficial, brought about in the germ plasm prior to amphimixis by extrinsic factors (in contrast to intrinsic germinal variation). A variety of chemical and physical agents are now known to be capable of exerting such an effect. It is obvious that it is in the male germ plasm particularly that blastophthoric processes can be recognized and studied, for postconceptional intra-uterine changes must be rigidly excluded. Moreover, much less is known about the normal and pathological histology of the ovary than of the testis.

There are three general methods by which injury to the male germinal epithelium may be investigated.

1. *The number and quality of the progeny* of supposedly altered germ plasm may be evaluated, a method much used in studies of human "race poisons." With human material the worth of the method may easily be overestimated. While presumptive evidence may be gathered in this manner, it is rare that results which can be considered proof can be obtained. The many interrelated variable factors make it impossible to develop such a statistical study in an entirely satisfactory manner. By adapting the same method to animal experimentation, however, the results may be greatly strengthened. By this method Stockard [3] established experimental proof of alcoholic blastophthoria and at the same time provided a model for the study of the blastotoxic effect of other agents.

2. *The number and quality* (*morphology, viability, vitality, motility, etc.*) *of the mature germ cells* may be compared after exposure to some extrinsic factor with the same attributes of germ cells under normal conditions. The exposure may be prior to maturation, after maturation but while yet within the parent body, or without the body but before fertilization has taken place. If variation from the normal can be demonstrated, it may be possible in experimental work to test the results of fertilization of, or by, such altered germ cells, in which case the method shifts to that first mentioned. In human material it is necessary to assume that cells so altered cannot

produce normal offspring. This assumption, however, is well supported by analogies from the field in which experimental test is possible.

3. *The processes of spermatogenesis and oögenesis* under the influence of extrinsic agents may be observed for alterations from the normal. As is well known, many agents when used intensively bring about complete aspermatogenesis through a process of degeneration of the germinal epithelium. It follows that in earlier stages of the process germ cells less seriously affected and still capable of fertilizing ova must have been produced. It must be realized that changes in the germ cells of such a nature as to give rise to markedly inferior offspring may be unrecognizable morphologically and that spermatozoa with demonstrable morphological changes may be incapable of fertilizing ova. That such marked changes are found as the end result of a continuous process of deviation from the normal justifies the use of this method, which is the one employed in the present study.

An abundant literature has accumulated in regard to the histological changes in the testis attributed to alcohol. This deals with both human and experimental material. In the former the earlier descriptions [4] were confused by the inclusion of changes now known to be due to syphilis, and it has rarely been possible to draw a sharp line between acute and chronic alcoholism in human material. It is interesting that the very frequent concomitance of syphilis and alcohol created the same difficulty in interpreting etiology in testicular pathology that still exists in regard to the liver. In experimental work these difficulties are avoided. The literature dealing with the testicular changes in both human and experimental alcoholism is briefly reviewed here in so far as it deals with relatively acute intoxication.

Review of Literature

Bouin and Garnier,[5] in 1900, examined microscopically the testes of two rats to which they daily had administered progressively increasing doses of diluted alcohol. The exact amounts used were not stated. While the length of time over which the experiment extended (8½ months in one instance and 11½ months in the other) was such as to make the word "acute" seem inappropriate, the results obtained were in accord with the pathological conception of

an acute, in contrast to a chronic, parenchymatous degeneration. Since the dosage was progressively increased it may be that the threshold of toxicity producing morphological change was not reached until late in the experiment. In one rat the testes appeared smaller and somewhat firmer than normal, in the other very soft. On section neither fibrosis nor vascular sclerosis was found. There was some diminution in the size of the tubules and varying degrees of reduction in the thickness of the germinal epithelium. There were but very few normal spermatozoa present but atypical free cells were found in the lumina. The disappearance of the seminal elements was found to be taking place in an order inverse to that of their origin. Pyknosis, chromatolysis and karyorrhexis of the nuclei; hyaline degeneration, plasmorrhexis and fatty change in the cytoplasm; vacuolar degeneration of both cytoplasm and nucleus, and the coalescence of spermatocytes or spermatids to form polynuclear plasmatic masses were described. They concluded that the seminal epithelium is highly vulnerable to prolonged ethyl alcohol intoxication, the more completely differentiated cells suffering more severely.

Todde,[6] in 1910, poisoned three cocks with large doses of ethyl alcohol. The first died in eight hours after the administration of about 50 cc. of twenty per cent ethyl alcohol. Its testes weighed 25 gm. and appeared of normal size and consistency. Of the second, which was killed after three successive daily intoxications, the testes were slightly smaller than normal, weighing 18 gm. The third was killed after four days and its testes were found to be much smaller than normal, weighing but 14.5 gm., although they showed nothing unusual in respect to color or consistency. Histological examination showed no marked changes. There was a little enlargement of the lumina of the tubules but "except for an indistinct and slight tendency to diffuseness all of the elements preserved a normal appearance and in most of the tubules, spermatogenesis was proceeding at its maximum intensity." It is difficult to reconcile these slight histological changes with an apparent reduction in weight to the extent of about 40 per cent as shown in the third instance.

Stockard and Craig,[7] likewise, failed to find changes of significance in the testes and ovaries of guinea pigs which had been treated with alcohol vapor for varying periods. In all cases the germ cells, both ova and spermatozoa, were found to exhibit an entirely normal structure.

Arlitt and Wells,[4] in 1917, made a detailed histological study of the testes of fifteen rats which had received from 0.25 to 2.25 cc. of ethyl alcohol daily for from two to ten months. Marked and nearly constant changes were found. A marked decrease in the size of the seminiferous tubules, leading in some instances to reduction in size of the testis itself, was shown by careful measurements with an ocular micrometer. The changes in the seminal elements appeared to take place in a definite order, inverse to that of the appearance of the various cell types. The first effect of the alcohol appeared to be to render the formation of spermatozoa incomplete, so that heads were formed without normal tails. This was further borne out by the fact that in such testes the number of spermatozoa in the epididymis was less than normal and proportionately less than the number seen in the seminiferous tubules. The next effect seemed to be to prevent the transformation of spermatids into spermatozoa, so that the tubules became filled with accumulated spermatids. The spermatids then degenerated, losing their power of nuclear staining and becoming granular. In the most advanced stages the tubules contained but marginal cells, with few or no spermatocytes or spermatids and occasional large cells with many nuclei free in the lumen. When the tubules were atrophic there was some compensatory edema *ex vacuo*. No inflammatory changes were seen and no marked fibrosis although in certain instances there had been a slight thickening of the basement membranes. The authors stated that the testicular changes found were in harmony with autopsy findings in human alcoholics. The ovaries of alcoholized females were examined also, but it was found impossible to make any positive statement as to alterations attributable to alcohol.

Kostitch,[8] in 1922, described the determination of an experimental alcoholic blastophthoria by histological study of the seminal epithelium. The testes of rats to which he had administered daily progressively increasing amounts of ethyl alcohol, from 0.5 to 3 cc. for from 17 to 120 days, were described in detail. Only his conclusions can be restated here: The seminal epithelium is especially sensitive to the action of alcohol. Its cells disappear in an inverse order to that of their genesis. At first the tubules become filled with masses of cells through desquamation of the more mature elements, with an accumulation of spermatids arrested in their development. Maturation divisions of the spermatocytes are disordered.

The accumulation of spermatids determines the formation of seminal teratocytes (teratospermatids). The arrest of spermatogenesis and the release of teratocytes reduces the epithelium to the primitive germ layer, the persistence of which makes possible a later regeneration. The sensitivity of the germinal epithelium to alcohol is much greater than that of the hepatic cells. Lesions of the germinal epithelium occur at a time when the hepatic cells show nothing. The lesions observed in the testis in the course of experimental alcoholic intoxication must be attributed directly to the action of pure ethyl alcohol. The nuclear changes in the germinal epithelium represent the essential phenomena of alcoholic blastophthoria. Short of being killed, the germinal epithelial cells exhibit alterations in their evolution. They may be arrested at any stage or may develop in an atypical manner leading especially to the formation of asymmetric mitoses with an unequal division of the chromosomes, a process in which there may be found an explanation of the occurrence of morphological defects in the children of alcoholics.

Apparently Kostitch was not familiar with the work of Arlitt and Wells, for he does not include it in his summary of the literature. The close agreement in the descriptions of the histological changes in these two detailed studies is significant.

Material

Nine coroner's autopsies were selected in which death occurred during, or immediately after, a period of severe alcoholic intoxication. No case with a massive pneumonia or other extensive acute infectious condition was admitted to the series. In using human material, unlike that from animal experimentation, it is practically impossible to maintain criteria any more rigid than those stated above. Contrary to the impression given by the daily press in regard to certain other clinical centers, we have fewer autopsies by far on cases of alcoholism than we did prior to national prohibition.

There are three objections to the proposed utilization of this material which must be discussed. The first is pertinent and unavoidable. In most instances it cannot be asserted that the terminal bout of drinking was not an exacerbation of an intermittent or more or less chronic alcoholism. To this extent the intoxication may not be strictly acute. On the other hand, the changes which are found

are of an acute degenerative nature, entirely comparable to those found in experimental animals. Outside of a rather narrow range, the exact length of time that the condition of alcoholism obtains seems to make relatively little difference in the picture. In every case used there had been a severe degree of intoxication shortly before death.

The frequency with which areas of syphilitic orchitis occur in the testes of these cases may raise a suspicion that the other changes which are to be described were due to syphilis and not to alcohol. There is no connection, and no resemblance, between the two pathological processes and, to one experienced in histopathological diagnosis, no possibility of confusion exists. Exactly similar patches of orchitis fibrosa syphilitica occur in the testes of men dying from a variety of conditions without the remainder of the parenchyma showing the degenerative changes found in the present group. Syphilitic changes in the testes are patchy in distribution and may be unilateral. Blastotoxic degenerations, such as are attributed to alcohol, are diffuse and bilateral. The syphilitic changes are primarily vascular; the alcoholic changes are primarily parenchymatous.

It will be noted that the complete autopsy examination showed various incidental conditions to be present in certain cases, particularly in the older men. These varying conditions can scarcely be important factors in causing the degenerative changes found in the testicular epithelium, which are all of the same general type and, taken together, constitute a unified retrogressive process. Moreover, in other autopsies all of these incidental disease conditions have been observed without accompanying alteration in spermatogenesis. The assumption of a causal relationship for alcohol in respect to the testicular degenerations described in these cases seems, therefore, to be justified.

CASE I. A boy, aged 17 years, was found dead in his automobile in the early morning after a drinking bout lasting part of the night. He was known to have been a user of alcohol before that time. The autopsy diagnosis was as follows:

Acute alcoholism. Congestion and edema of brain with cloudy swelling of ganglion cells. Congestion and edema of meninges with minute petechial hemorrhages in brain and meninges. Severe acute degenerative catarrhal gastritis. Extreme congestion and edema of all organs. Acute fatty degenerative infiltration of heart and liver.

Acute lipoidosis of adrenals. Acute cloudy swelling of kidneys. Lymphatic constitution.

Microscopic examination of the *testes* showed tubules which appeared unusually large, the lumina being dilated without thinning of the wall. On the contrary there was a moderate, but definite increase in the number of spermatocytes and spermatids present. Apparently normal spermatozoa were found in most of the tubules. The appearances suggested a retardation in the evolution of the various orders of cells of such a nature that the total number of spermatocytes and spermatids present at one time was increased. Numerous division figures occurred some of which may have been atypical although this cannot be positively asserted. No teratocytes or giant nuclear forms were seen.

CASE 2. A laborer, aged 42 years, was said to have consumed an enormous quantity of homemade wine, fortified with some form of "bitters" or "wine of beef and iron," during the five days before his death. There had been severe diarrhoea and vomiting and he was said to have fallen downstairs once during the period of his intoxication. The final pathological diagnosis was:

Acute exacerbation of chronic alcoholism. Chronic leptomeningitis. Acute encephalitis. Atrophy, passive congestion and edema of brain. Acute toxic gastro-enteritis on an older chronic catarrhal inflammation. Chronic esophagitis. Fatty heart. Polypoid thrombus in right ventricle. Atherosclerosis. Chronic passive congestion of lungs and acute purulent bronchopneumonia (aspiration pneumonia). Chronic parenchymatous degenerative nephritis. Fatty degenerative infiltration of liver. Acute purulent prostatitis with multiple abscesses. Healed tuberculosis of apices and spleen. Traumatic abrasions of skin. Hypertrophy of adrenals. Atypical spermatogenesis.

In addition to the evidences of acute and chronic alcoholism various incidental findings were encountered in this instance. The terminal aspiration pneumonia involved but small areas in the lower portion of each lung. It seems improbable that it could have been responsible for the testicular change. The nephritis was old and of a very moderate degree. The cardiac failure was an end phase of the alcoholic bout. It seems proper, therefore, to attribute the acute degenerative changes primarily to alcohol.

Microscopically, the *testes* showed as the most striking feature an accumulation of spermatocytes and spermatids of so marked a de-

gree as nearly to fill the lumina of the tubules. These cells were not free but still in position in the epithelium. In areas the picture was that of solid cords of cells. A few normal appearing sperm cells were present in most of the tubules. An occasional giant nucleus was found in the zone of spermatogonia, but no free teratocytes. Vacuolar degeneration was lacking. There was a very slight diffuse thickening of the basement membranes.

CASE 3. A farmer, about 50 years old, sold a load of produce, bought and drank a large quantity of whisky and was put upon his wagon while drunk by his companions. When his team of horses reached home he was found dead in the wagon. The pathological diagnosis was as follows:

Acute alcoholism. Congestion and edema of brain. Fatty degenerative infiltration of heart and liver. Acute congestion of all organs. Chronic catarrhal gastro-enteritis. Early sclerosis. Prostatic hyperplasia.

Microscopic examination of the *testes* showed a few spermatozoa in many tubules. The lumina of the tubules were dilated and the epithelium of very uneven thickness, in many areas being definitely reduced. Small masses of desquamated spermatids, apparently agglutinated, were found in many tubules. Of the spermatogonia and spermatocytes some showed an early vacuolar degeneration of the cytoplasm. Occasional cells, apparently spermatogonia, with giant nuclei up to three times the normal diameter in size, were found resting upon the basement membrane. No free teratocytes were noted. There was no increase in the stroma.

CASE 4. A brick mason, 62 years old, was found dead in his room. The coroner's diagnosis was "acute alcoholism." The final pathological diagnosis was:

Acute exacerbation of chronic alcoholism. Chronic leptomeningitis. Congestion and edema of brain. Cloudy swelling of ganglion cells. Chronic atrophic catarrhal gastritis, alcoholic. Old syphilis. Chronic fibroid myocarditis. Brown atrophy of heart. Early sclerosis of aorta. Chronic passive congestion of lungs with stasis, edema and anthracosis. Healed tubercles in lungs with calcareous tubercles in bronchial nodes. Chronic interstitial pancreatitis. Lipoidosis of adrenals. Chronic passive congestion and atrophy of all organs. Atypical spermatogenesis. Areas of interstitial fibrosis in testes. Rhabdomyoma of kidney.

The changes incident to advancing years and an old latent syphilis were found in this instance in addition to evidences of alcoholism. The acute degenerative changes shown microscopically in the testes could not be due to either of the former. The teratoid neoplasm in the kidney was of small size and of no clinical importance.

The *testes*, upon microscopic examination, showed a few spermatozoa in many tubules. There were also numerous masses of free cells, chiefly spermatids and spermatocytes which were apparently agglutinated. The tubules were dilated, the size of the lumina being increased in part by the marked thinning of the epithelium which was frequently found reduced to two or three layers of cells. In selected tubules there was noted a well marked vacuolar degeneration of the epithelium. This change showed a zonal distribution involving particularly the spermatocytes with a zone of nearly normal-appearing spermatids internal to the region of vacuolation. Among the free cells in the lumina teratocytes occurred in small numbers. These were chiefly multinucleate masses with abundant pink-staining cytoplasm containing from four to eight or more nuclei of spermatid or even sperm-head type. These masses gave the impression of having been formed by nuclear divisions unaccompanied by division of the cytoplasm. Cells of spermatocyte type with two or four nuclei were found in small numbers embedded in the wall. Giant nuclei were numerous. There was a slight diffuse increase in thickness of basement membranes and a few small groups of tubules showing complete fibroid obliteration of syphilitic type.

CASE 5. A mulatto porter, aged 33 years, who was known to have been intoxicated for two days, was found dead in a toilet-room. The coroner's diagnosis was "acute alcoholism." The autopsy diagnosis was as follows:

Acute alcoholism. Marked acute catarrhal gastro-enteritis. Congestion, edema and parenchymatous degeneration of all organs. Multiple hemorrhages in lungs. Syphilis (leptomeningitis, active aortitis, adrenalitis, orchitis). Persistent thymus. General lymphoid hyperplasia. Small esophageal papilloma.

Microscopic examination of the *testes* showed a greatly reduced number of spermatozoa, but a few normal appearing sperm cells were found in many tubules. There was extensive desquamation of both spermatids and spermatocytes with masses of agglutinated cells blocking the lumina of certain tubules. The epithelium was

correspondingly thinned and showed a moderate vacuolar change with no sharp zonal localization. The only teratocytes found were deep in the epithelium, near the basement membrane, and consisted of large cells with three to four nuclei of spermatocyte type. A few cells with single giant nuclei were noted also. There was no general fibrosis, but a few small patches of fibroid atrophy of syphilitic type.

CASE 6. A laborer, whose apparent age was about 55 years, was found dead. The stomach contents had still a strong odor of whisky at the time of autopsy. The pathological diagnosis was as follows:

Acute exacerbation of chronic alcoholism. Chronic syphilitic myocarditis, aortitis, pancreatitis and orchitis. General arteriosclerosis. Atrophy, passive congestion and parenchymatous degeneration of all organs. Early hepatitis. Lipoidosis of adrenals. Asphyxia. Hypernephroma.

The *testes* showed microscopically numerous apparently normal spermatozoa. The tubules were slightly dilated and in certain regions the number of spermatocytes and spermatids was increased to a very slight degree. Occasional tubules showed a rather sharply localized vacuolar change. A few teratocytes were found and only a few nuclei which definitely exceeded the normal range in size. There was a slight diffuse thickening of the basement membranes but no fibrosis of syphilitic type.

CASE 7. A young man who appeared to be about 30 years old, was placed under arrest while in an active alcoholic delirium. Chloral hydrate was administered to quiet him, the drug being given in more than ordinary therapeutic amount. He was found dead in his cell the following morning. The pathological diagnosis was as follows:

Acute alcoholism. Chloral hydrate poisoning. Passive congestion and stasis of all organs. Edema of lungs. Chromatolysis of cortical cells. Lipoidosis of adrenals. Early sclerosis of aorta. Excessive hemolysis.

Microscopic examination of the *testes* showed very few spermatozoa. The lumina of the tubules appeared dilated, largely because of the thinning of the epithelium. There was a well marked vacuolar change in both cytoplasm and nuclei, especially the latter. In certain tubules the vacuolar change occupied an intermediate zone and in a few the apparent transposition of the order of spermatogenetic cells, noted by Kostitch, was seen. In these areas a row of sper-

matids was found external to a zone of spermatocytes of the second order. Apparently these spermatocytes had suffered an arrest of development while the epithelium beneath them had brought on a new generation of spermatids. Free teratocytes, masses of cytoplasm with multiple nuclei, were found in small numbers. Usually two or four nuclei were present in each; in one, twelve could be counted. There were a few giant nuclei. There was no increase in the stroma.

The possibility that chloral hydrate poisoning was a factor in producing the changes found cannot be entirely excluded. It will be recognized, however, that the deviation from the normal is more marked than in any of the previously described cases. Not more than five or six hours had elapsed between the administration of this drug and death. It is impossible that the changes found could have been produced entirely within that interval, although they may have been intensified.

CASE 8. A farmer, formerly a miner, had been drinking heavily for some time and was intoxicated during the day and evening preceding his death. He went to sleep in a barn with a companion and was found dead the next morning. The autopsy yielded the following diagnosis:

Homicide. Fracture of intervertebral disc between sixth and seventh cervical vertebrae. Laceration of cord. Fresh traumatic ecchymoses of skin and cervical tissues with massive, fresh, retropharyngeal hematoma. Hemorrhagic suffusion of all mediastinal tissues. Death by asphyxia. Pulmonary stasis, edema and petechial hemorrhages. Old healed tuberculosis of lungs and of bronchial, mediastinal, mesenteric and retroperitoneal lymph nodes. Old adhesive pleuritis. Coronary sclerosis. Atrophy of myocardium. Epicardial sclerosis. Atherosclerosis of aorta. Chronic catarrhal gastro-enteritis. Acute exacerbation of chronic alcoholism. Atrophy, acute passive congestion and parenchymatous degeneration of all organs. Obliterated appendix. Old vesiculitis. Glandular hyperplasia of prostate. Old infarcts in renal cortex. Hypertrophic spondylitis of the lower thoracic vertebrae. Lipoma of perineum.

Death must have occurred in a relatively short time after injury to the cervical vertebrae and spinal cord. The changes described for the testes were of an acute zonal character and could not have been produced by the asphyxia resulting from that injury.

Microscopically the *testes* showed active spermatogenesis with normal appearing spermatozoa in many tubules. The lumina of the tubules were somewhat dilated and contained many desquamated spermatids and spermatocytes both singly and in clumps. A few multinucleate forms were included among these. The greatly thinned epithelium showed a well marked vacuolar change. Giant nuclei in small numbers were noted. In addition to a slight diffuse thickening of the basement membrane there were patches of fibrosis of the syphilitic type.

CASE 9. A man, aged 44 years, was found unconscious in his bed. He was taken to the hospital, but died within twenty-four hours without regaining consciousness. The reflexes were unaltered and there was no paralysis. There were no marks of violence upon the body. Aside from the coma, frequent diarrheic stools were the only symptom. The diagnosis as determined at autopsy was as follows:

Acute exacerbation of chronic alcoholism. Beer drinker's liver, early stage. Chronic parenchymatous nephritis of moderate degree. General sclerosis. Acute and chronic passive congestion of all organs. General atrophy. Lipoidosis of adrenals. Syphilitic atrophy of testes. Chronic leptomeningitis.

Upon microscopic examination the *testes* showed almost complete aspermatogenesis. In only two tubules were normal-appearing spermatozoa found. There were but few free cells and the greatly thinned epithelium showed a marked vacuolar change. In certain tubules this was zonal in position, affecting spermatocytes most severely while a single row of spermatids near the lumen showed but little change. Neither teratocytes of the multinucleate form nor giant nuclei were found. The changes in the germinal epithelium were the most marked of any in the series. Numerous patches of old syphilitic fibrosis and a slight diffuse thickening of the basement membranes occurred.

In Table I the chief histological changes in the testes of these nine cases are summarized. The cases have been described above in an order which was determined in so far as possible by the duration of the acute alcoholism. In some instances relatively little was known in regard to this point, but the history and the general autopsy findings have been taken together somewhat arbitrarily to determine this order. In Table I, however, the order has been determined by the stage and degree of severity of the testicular changes alone.

TABLE I

Summary of Microscopic Findings with Cases Arranged in Order of Increasing Severity of the Degenerative Process

	Case Numbers								
	1	2	6	3	4	5	8	7	9
Normal appearing spermatozoa present	++	+	++	+	+	+	+	±	±
Lumen occupied by masses of free cells	−	−	−	+	+	+	+	−	−
Tubules dilated	+	+	+	+	+	−	±	+	±
Epithelium thinned	−	−	−	+	+	+	+	+	+
Spermatids increased	+	++	±	−	−	−	−	−	−
Spermatocytes increased	+	++	±	−	−	−	−	−	−
Vacuolar degeneration of cytoplasm	−	−	±	+	+	+	+	+	++
Vacuolar degeneration of nuclei	−	−	−	−	±	−	−	+	+
Zonal degenerative changes	−	−	±	−	+	−	−	±	+
Teratocytes	−	−	−	−	+	+	±	+	−
Giant nuclei	−	+	±	+	+	+	+	±	−
Diffuse fibrosis of basement membrane	−	±	±	−	±	−	+	−	+
Fibrosis of syphilitic type	−	−	−	−	+	+	+	−	+

Some degree of parallelism exists between the two arrangements. This might have been closer if more accurate histories had been available.

Spermatozoa: Normal appearing spermatozoa were found in every case, although they were very few in two instances. This material had been fixed and stained by routine methods only (chiefly formol fixation with hemalum and eosin staining) so that finer morphological deviations could not be adequately investigated. Judgment had to be based very largely upon nuclear form and chromatin content. In spite of the marked degenerative changes present spermatogenesis had taken place recently or was still taking place in every instance. This supports the belief that the processes here described

are essentially acute in nature, and leaves open the possibility of impregnation during the stages of degeneration represented in this group.

Increase in Spermatids and Spermatocytes: In the first, and to a more marked degree in the second case, the increase in spermatids and spermatocytes was striking. Many tubules appeared as solid masses of cells, the lumen having been encroached upon so much as to be practically obliterated. This apparent retardation of spermatogenesis with resulting accumulation of immature forms occurred only when evidences of more severe degenerative changes were lacking. It must be considered one of the earlier manifestations of this type of injury.

Desquamation and Reduction of the Epithelium: With somewhat more severe injury extensive desquamation of the inner layers of the thickened epithelium occurred and free spermatids and spermatocytes as well as clumped aggregates appeared in the lumina. With this process vacuolation of the remaining epithelium was usually present. The epithelium was reduced to two or three layers of cells in those tubules with marked desquamation. This produced the appearance of dilatation of the tubules themselves due to the wide lumina. While no accurate measurements were attempted because of lack of standardization in fixation it was evident that the tubules themselves were actually somewhat smaller than normal.

Vacuolar Degeneration: Vacuolar degeneration of the cytoplasm was found in all of the more severely injured testes. This vacuolation is hydropic in nature and is part of a process leading in its most advanced stages to liquefaction necrosis. In three instances small vacuoles were found within the nuclei also. An interesting picture was presented by the four cases in which the vacuolation occurred with a zonal distribution, affecting spermatocytes or spermatogonia more severely than the spermatids nearer the lumen.

Atypical Forms: Without special methods the more minute deviations from normal in karyokinesis cannot safely be described. That such must have been present was shown by the grosser variations in morphology which were constantly encountered when degenerative changes were marked. These were chiefly of two sorts. In the basal layer hyperchromatic cells, some with enormous single nuclei which have been called "giant" nuclei were frequently found. In the lumen, and also still attached to the wall, multinucleate

"teratocytes" were frequently encountered. These had the appearance of being formed by repeated nuclear divisions without corresponding fission of the cytoplasm. Especially was it true of those with many nuclei that the amount of cytoplasm present was insufficient to sustain the belief that the teratocyte had been formed by the fusion of previously separate units.

Fibrosis: In four of the nine cases patches of fibrous orchitis of syphilitic type were found. It has previously been pointed out that this condition has nothing in common with the changes which are attributed to alcohol and cannot be confused with them. In three instances there was a slight, and in two a moderate, diffuse fibrosis of the basement membranes. It is possible that there may be a relationship between this and chronic alcoholism but at present there is no evidence by which this can be either affirmed or denied.

Lack of Specificity: The changes which are here attributed to acute alcoholism are in no sense specific for that condition. There are apparently many extrinsic factors which can produce similar degenerative processes. We have been able to duplicate every aspect of them in experimental lead poisoning in guinea pigs, and Mills [9] has described similar changes associated with pneumonia. Neither is there proof that alcohol acts directly upon the germinal epithelium. The immediate damage may be elsewhere, perhaps in the liver, and the testicular effect secondary.

Blastophthoric Significance: The significance of such a marked degenerative process as was found in the testes of these cases of acute alcoholism is evident. The changes described exceed in degree those which are produced in the course of the experimental demonstration of alcoholic blastophthoria by means of breeding experiments. It is certain, in view of the atypical cell forms and the marked degeneration of both cytoplasm and nuclei, that abnormal spermatozoa are produced. This demonstrates, therefore, a morphological basis for experimental alcoholic blastophthoria, for prior to the development of such marked degenerative changes, spermatozoa capable of giving rise to defective offspring must be set free. Procreation during a period of intoxication thus entails a definite hazard as to the quality of the offspring which may result. There is experimental evidence to show that the basal layer of cells in the tubular epithelium (primitive germinal epithelium) suffers the least and is capable of regenerating a new series of spermatogenetic cells.

SUMMARY

In the testes of nine men who died in a period of acute alcoholism, parenchymatous degeneration leading, when sufficiently marked, to practically complete aspermatogenesis was found. In the testes showing less severe changes there was an increase in spermatocytes and spermatids with a decrease in spermatozoa. As a more severe change this was followed by desquamation releasing free cells and masses of cells in the lumina of the tubules. Vacuolation of the cytoplasm, sometimes zonal in character, and in severe cases vacuolation of the nuclei, occurred. The vacuolated epithelium became reduced to but one to four layers of cells, only the basal layer being intact. Atypical cell divisions producing hyperchromatic giant nuclei and multinucleate teratocytes were numerous.

These observations place clinically recognized, and experimentally induced, alcoholic blastophthoria upon a morphological basis.

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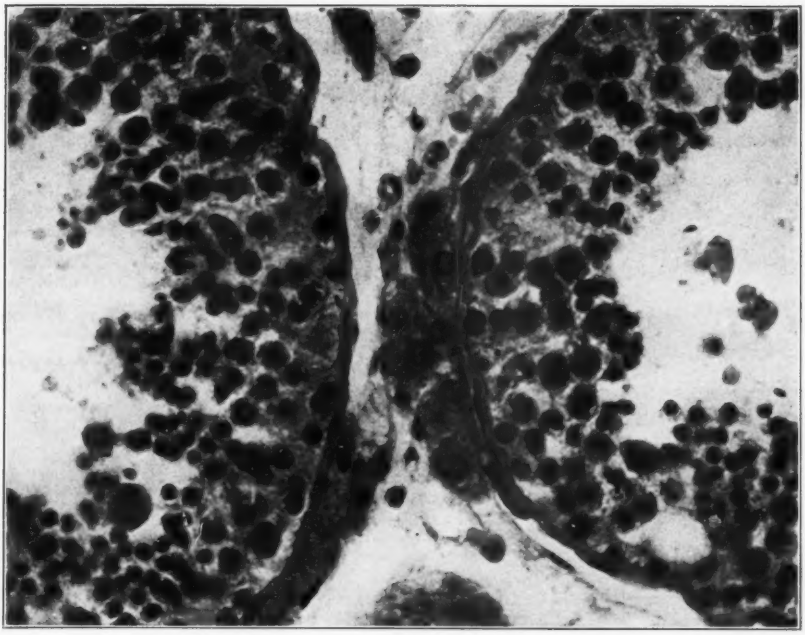
DESCRIPTION OF PLATES

PLATE 1

FIG. 1. Case 1. The early effects of acute alcoholism upon spermatogenesis. Spermatocytes and spermatids increased in number, spermatozoa decreased in number. × 465

FIG. 2. Case 2. Marked increase in number of spermatocytes and spermatids with reduction in spermatozoa. Cell masses nearly occlude the lumina of the tubules. × 325



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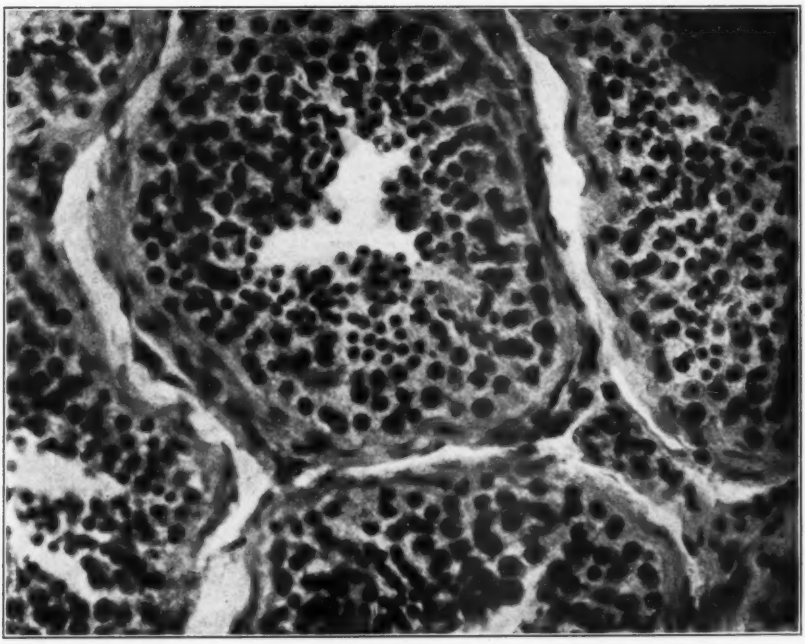

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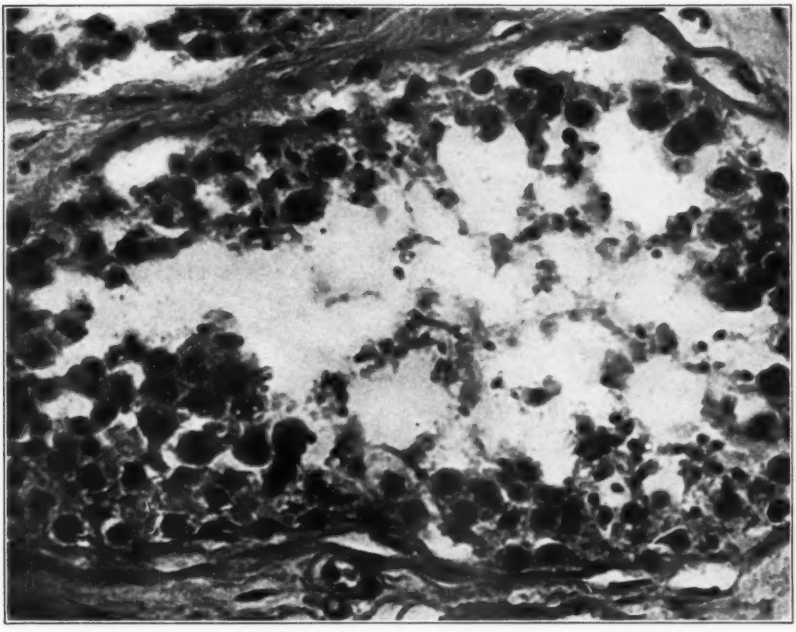
Weller Male Germinal Epithelium in Acute Alcoholism

Plate 2

Fig. 3. Case 3. Reduction of number of layers of epithelial cells by desquamation and vacuolation. Sperm heads with cellular débris in the widened lumen. × 465.

Fig. 4. Case 4. Marked thinning of the epithelium with desquamation of spermatids and spermatocytes. Giant nuclei among the spermatocytes and also in the basal layer of the epithelium. × 465.



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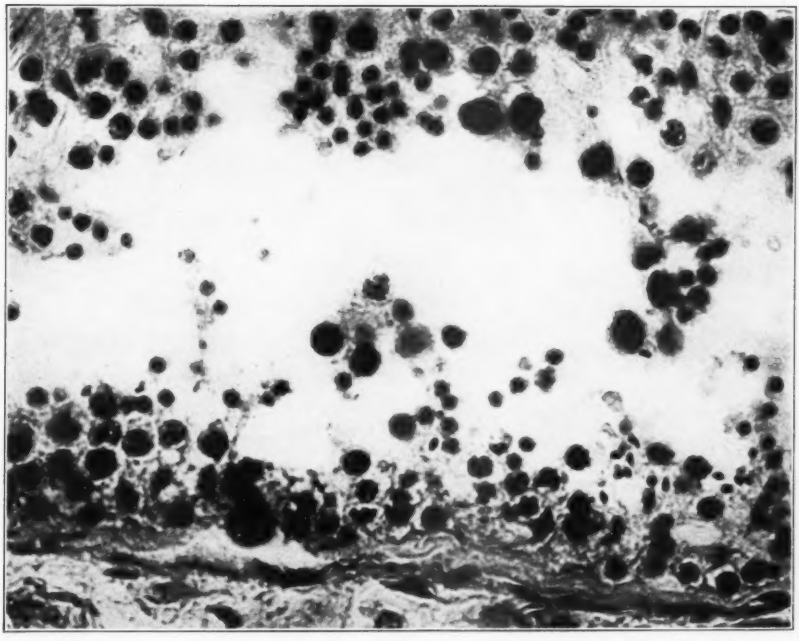

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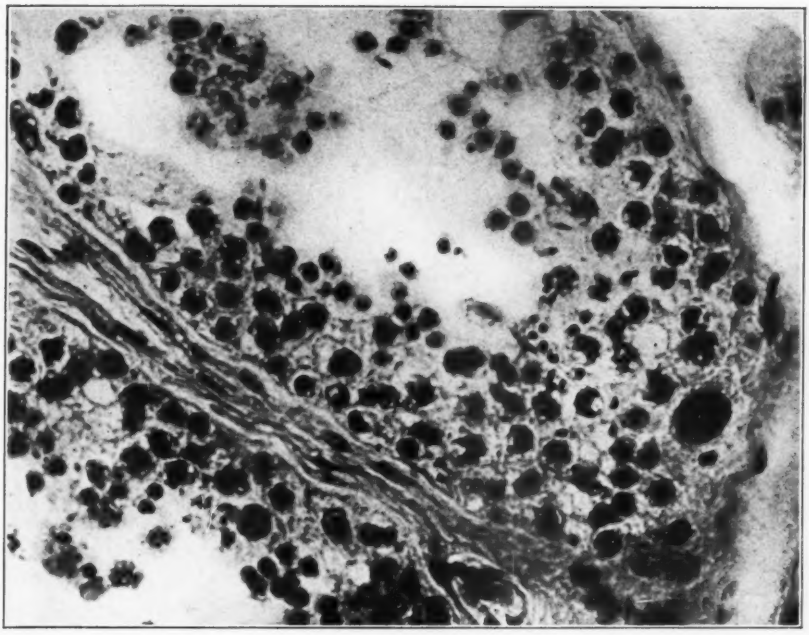
Weller Male Germinal Epithelium in Acute Alcoholism

Plate 3

Fig. 5. Case 4. Vacuolar degeneration of the epithelium with desquamation. Hyperchromatic giant nucleus in the basal layer. × 465.

Fig. 6. Case 5. Desquamation of spermatids and spermatocytes, filling the lumen with cells. Marked vacuolar degeneration of remainder of germinal epithelium. × 465.



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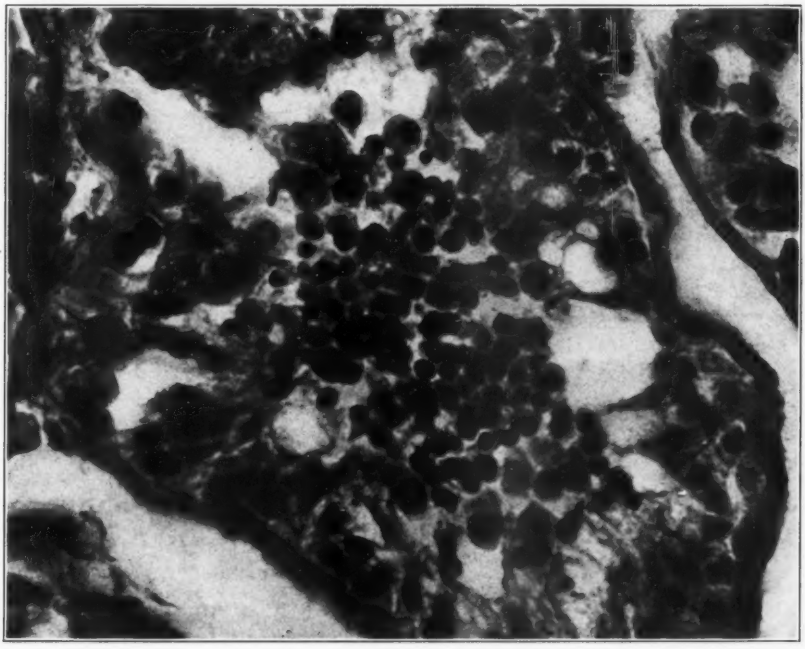

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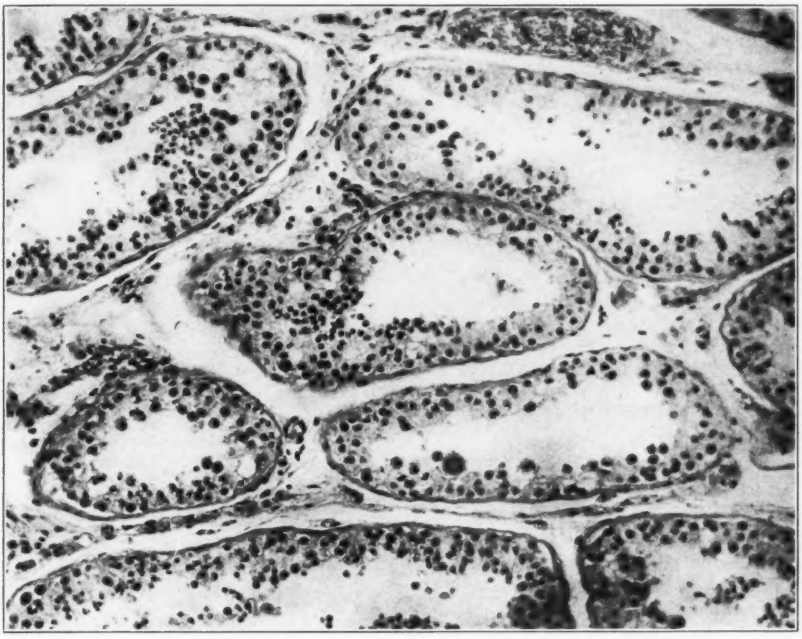
Weller Male Germinal Epithelium in Acute Alcoholism

PLATE 4

FIG. 7. Case 6. Multinucleate teratocyte in tubule in lower center of field. × 130.

FIG. 8. Case 6. Vacuolar degeneration and desquamation, these changes being more severe in this tubule than was general for this testis. × 465.



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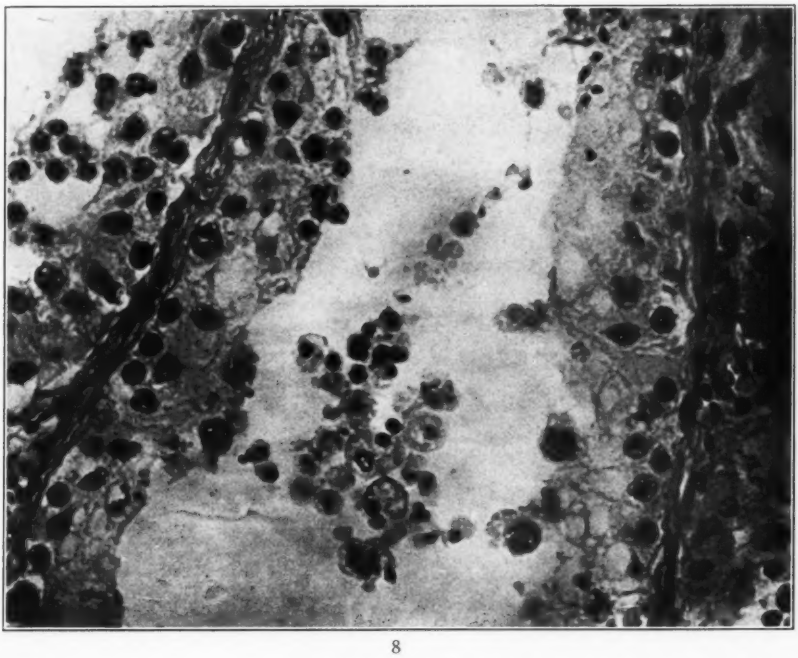

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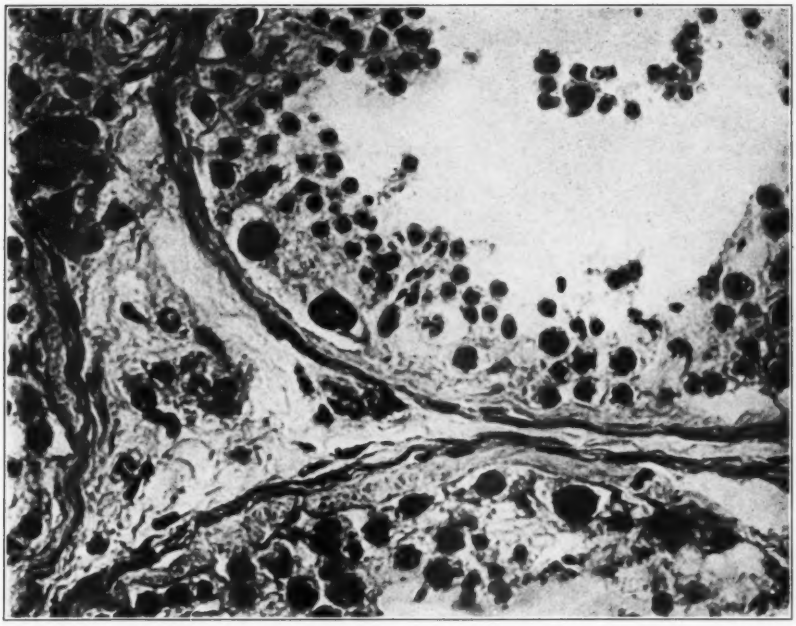
Weller Male Germinal Epithelium in Acute Alcoholism

Plate 5

Fig. 9. Case 7. Hyperchromatic giant nuclei in the basal layer of the epithelium. × 465.

Fig. 10. Case 8. Vacuolar degeneration and desquamation. Multinuclear teratocyte in upper portion of field. × 465.



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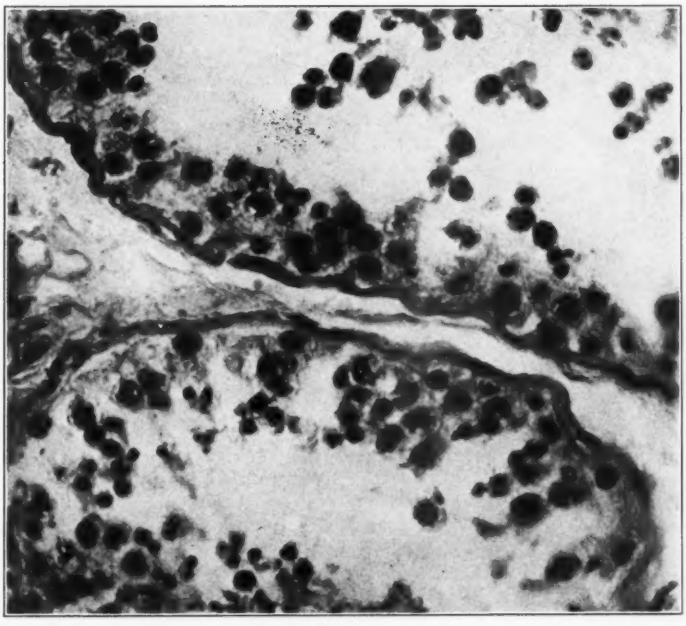

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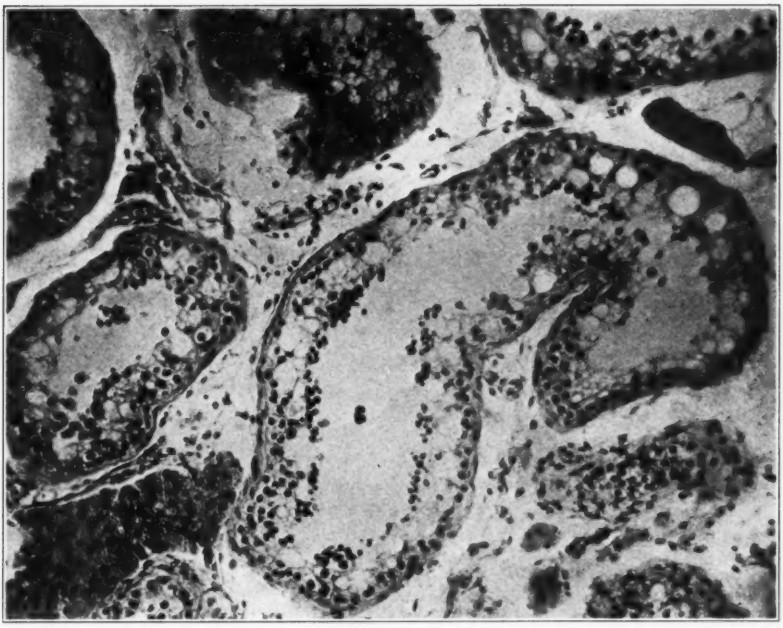
Weller Male Germinal Epithelium in Acute Alcoholism

Plate 6

Fig. 11. Case 9. Lower power to show extreme degree of vacuolar degeneration of spermatocytes with marginally disposed non-vacuolated spermatids. × 140.

Fig. 12. Case 9. Zonal vacuolar change with non-vacuolated spermatids and spermatocytes adhering to the wall. Numerous hyperchromatic nuclei, but not of "giant" size. × 465.



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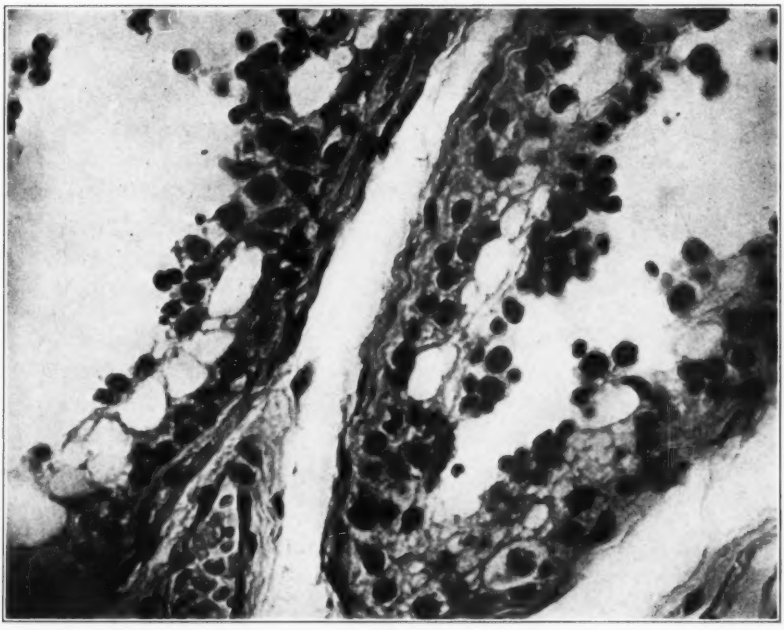

12

Weller Male Germinal Epithelium in Acute Alcoholism

THE VENOUS DRAINAGE OF THE CAT SPLEEN *

W. L. Robinson, B.A., M.B.

(From the Department of Pathology, University of Toronto, and The Toronto General Hospital, Toronto, Canada)

As has been established in a previous communication,[1] the circulation through the spleen is an open one. The arterial system terminates in a widely distributed series of capillaries which in the dog, cat and sheep open out in a bell-shaped manner, and in the human in ampullary dilatations with oval-shaped apertures to discharge their contents into the pulp spaces. The pulp which represents the functional part of the spleen is formed by a vast network of reticulo-endothelial cells (pulp cells), supported by delicate threads of reticulin. The general construction is such that the elements of the blood are brought into intimate contact with the pulp cells, which have been shown by the author [2] to play an important part in the filtrative function exerted by this organ.

From the above findings it is obvious that the venous system is independent of the arterial system and manifests its chief function by draining the pulp spaces of their contents. Mall [3] has shown the emptying of the spleen is largely brought about by a contraction of the trabecular framework, which by virtue of its attachments to the walls of the veins pulls them open and at the same time compresses the pulp. During the contractions of the capsule and trabeculae a pressure may be built up in the venous system which is higher than that on the arterial side. Under these circumstances the blood no doubt is prevented from flowing back into the arteries by the concomitant compression of the ellipsoids with that of the pulp. The ellipsoids therefore function as check-valves for the arterial system. The rate of flow of blood through the normal dog spleen is not very great, being, according to Mall about 5 cc. per minute. I have found in perfusing cat and dog spleens with a pressure head of seventy-two inches of water on the arterial side and a back pressure of ten inches on the venous side, the spleen being fully distended, that the outflow of fluid from the vein averages 6 cc. per minute. This is fairly con-

* Received for publication July 5, 1929.

stant. On the other hand when the spleen is fully distended with the perfusing fluid and the back pressure on the venous side released, there is a sudden flow of the fluid from the vein out of all proportion to the rate of inflow. This keeps up until the pulp system is partially emptied. Following this the rate of outflow becomes normal, being as in the first case, the same as the arterial inflow. It is seen therefore that there is a considerable disproportion between the rate of inflow and the possible rate of outflow. Barcroft [4] has shown that considerable variations in size of the spleen may occur in the living animal. This he considers to be an emergency measure for the sudden flooding of the circulation with stored up blood elements from the spleen. These observations indicate a very free and intimate connection between pulp spaces and venous channels, much more so than between arterial system and pulp, else why the rapid emptying of the spleen as compared with its filling time? The communicating channels between the pulp spaces and the veins must therefore be numerous and of generous proportions. The study of this relationship of the venous system to the pulp in the cat spleen represents the subject matter of this paper.

The venous sinuses are readily recognized in sections of distended spleens, particularly in those of dogs and humans. In the latter their cubic content appears to be almost as great as that of the pulp itself. In his excellent phylogenetical study of the venous capillaries of the spleen Mollier [5] found that while there were certain fundamental principles of structure common to all, variations in the character of their walls in the different species were manifested. In the more primitive types the pulp tissue itself is used unchanged in this bounding process, the veins being nothing more than a continuous series of pulp spaces of uniform size. The higher types have more clearly defined walls made up of a specialized layer of endothelium. He has made an exhaustive study of these variations in structure and in all of them, even in the most highly developed, he has shown that their walls are incomplete. Fenestrations or stomata are present which communicate with the pulp spaces to allow for the ebb and flow of the blood elements between the venous capillaries and the pulp. The bulk of his discussion centered about the presence or absence of these openings in the capillary walls and the character of the lining cells which would allow for such fenestrations. Apart from the discussion on the structure of the walls of the veins, one would gather from his

paper that he believed the arterial and venous systems to be a unity, for he states that the venous capillaries "do not form an independent system but are merely canals (Gänge) formed in the pulp tissue." He believed the spleen to have a closed circulation except for the ebb and flow, as described above, through the sieve-like walls of the venous capillaries. This conception however in the light of our findings is untenable. The arterial capillary system is independent and is separated from the venous system by the pulp spaces. Having identified and described the terminations of the arterial system in the pulp I was anxious to demonstrate the beginnings of the venous system. As far as dog, human and sheep spleens were concerned I was unsuccessful. Recently however while examining sections of distended cat spleens with a Zeiss binocular microscope with stereoscopic attachments, I was able to identify definite expansions of many of the venous capillaries which I concluded were the beginnings of this system.

Fresh cat spleens were used. The collateral circulation was carefully tied off and cannulae inserted into the splenic artery and vein. The spleen was taken out and placed in warm normal physiological saline. To avoid the possibility of tearing the delicate structures of the pulp tissue it was found advisable first to perfuse the spleen by way of the artery with warm normal physiological saline. A pressure head of about seventy-two inches (water), on the arterial side was found necessary to establish the flow of the perfusing fluid. Finally before the spleen could be completely distended it was necessary to produce a certain amount of back pressure on the venous side. This was done by attaching a piece of rubber tubing to the cannula in the vein and raising the outlet ten inches above the spleen. When the spleen was fully distended and the return flow of fluid from the vein clear, the physiological saline supply was cut off and the cannula in the artery connected to a supply of Zenker's fluid at the same pressure head. The perfusion of Zenker's fluid was allowed to continue until all the tissues were thoroughly bathed in the fixative and the saline well replaced by the fixing fluid. The vein was then clamped off and the saline in the dish about the spleen replaced by Zenker's fluid. The spleen was then left with a slightly reduced pressure head of Zenker's fluid still maintained on the arterial side for some two or three hours. The artery was then clamped and the spleen left for twenty-four hours in the fixative. Blocks were carefully cut with a

sharp knife, washed, dehydrated and embedded in paraffin in the usual manner.

The spleen in its distended state is very spongy. It was found therefore advantageous to cut the sections at 20 to 25 microns. The thin filmy membranous walls of the veins in the cat spleen are very difficult to demonstrate unless properly stained. The most satisfactory stain, I found, was Heidenhain's iron hematoxylin, staining deeply and differentiating only sufficiently to take out some of the excess stain. No counterstain was found necessary or advisable. The minute structures of the venous system could then be demonstrated in a very striking manner in stereo with the Zeiss binocular microscope.

Tracing the venous system backward from the hilum, the large collecting veins before entering the spleen were found to be similar in structure to those in other parts of the body. Their wall consisted of an inner lining of endothelial cells supported by a thin muscle coat and outside of this a layer of loose areolar connective tissue. They were closely approximated by the branches of the splenic artery, artery and vein usually entering the spleen together. The general distribution of the arteries and veins throughout the spleen was, I found, as described by Mall.[3] That is, the veins and arteries tended to diverge and occupy positions within the splenic lobules quite separate from one another.

The entrance of the veins into the spleen usually occurred at a point where the trabeculae formed their attachment to the capsule, the capsule here being slightly invaginated. I was able to find however at certain points between trabecular attachments to the capsule that small branches of the hilum veins directly penetrated the capsule at an angle to open out into the adjacent pulp tissue. This however was not the usual finding. Of the three coats just described, only one, the inner lining layer of endothelial cells was found to continue on into the spleen. The muscle coat and adventitia stopped abruptly at the capsule. From this point on, the venous system through the spleen was found to be capillary in nature, consisting of a series of branching channels whose walls were made up of a single layer of endothelial cells. On entering the spleen the veins first pursued a course in the center of the trabeculae for variable distances. These represented the interlobular veins described by Mall. They were supported directly by the longitudinal muscle, elastic, and con-

nective tissue fibers of the trabeculae. A few short branches were given off to the surrounding pulp tissue. These were similar to the terminal branches and will be described later.

Tracing farther the interlobular veins in their course within the trabeculae one found that they very soon veered off into the pulp tissue proper. They are supported now only in part by the trabecular fibers. Looking at them in cross-section one finds that this support is anywhere from a small fraction to almost the whole of their circumference. Where supported, the endothelial cells are lying directly upon the trabecular fibers. Where unsupported, the walls consist merely of a single syncytium-like layer of endothelial cells in direct contact with the neighboring pulp cells. In the unsupported portions I was able to observe rounded or oval-shaped stomata which communicated directly with the neighboring pulp spaces. Their margins in many cases were everted, giving them an appearance very much like short, side branches which expand out in a bell-shaped manner to communicate with the pulp spaces. They were however not numerous and must play but a minor part in the drainage of the pulp as a whole.

Following the veins into the pulp I found that they soon became completely independent of the trabecular fibers as a supporting structure. Frequently however attachments between them were seen. After running a course of not more than 0.3 mm. the final branching occurred. These vessels being independent of the trabeculae and lying wholly within the lobule were called by Mall the intralobular veins. In their first part they consist of channels having thin walls made up of a single layer of syncytium-like endothelial cells supported directly by reticulum and the neighboring pulp cells. Their nuclei are oval and somewhat flattened. They are comparatively few in number. They appeared to have no definite arrangement except that their long axis was the same as that of the vessel. Their cytoplasm was drawn out into thin protoplasmic sheets, one cell blending with another in such a manner that it was impossible to distinguish them. Numerous rounded or oval-shaped stomata were found in their walls, very similar to those previously described.

Finally with the next system of branching, the end of the venous system was reached. These, functionally, are probably the most important branches. They vary from 0.1 to 0.5 mm. in length. Their walls in the first part are fairly well defined but as they reach the

terminus they end in such an indefinite manner that they are almost indistinguishable from the pulp cells. They are similar in structure to the branches just described but their stomata are larger and more numerous. As one traces a branch to its terminus one finds that the stomata become larger, gradually approximating those of the adjoining pulp spaces. In this way the stomata gradually become pulp spaces and the bounding cells, pulp cells. The terminus therefore may be very difficult to identify. Sometimes however the branches seem to end in a sort of an ampullary dilatation with numerous stomata opening out in all directions. Again one might find the walls gradually converging to terminate at a point. The stomata as before become larger as this point is reached. In all cases these branches were found to terminate in the pulp. I was unable to identify any direct communicating channels between artery and vein.

As we have been tracing the system backward the branches just described as terminal are, in truth, the beginnings of the venous system. These, I think, might be called the primordial branches to distinguish them from others which are more or less collecting channels. The obvious course of the blood flow from the pulp to the venous system is through the large stomata in the primordial branches. A certain amount, of course, must also flow directly from the pulp through the stomata of the intralobular veins and interlobular veins where not ensheathed by the trabeculae.

Mollier's explanation of the circulation through the spleen as being through a continuous system of tubules is therefore wrong. The venous system is independent as is also the arterial system. His description and study of the phylogenetical development of the venous capillaries is very complete and I quite agree with his conception of the structure of their walls as seen in the various animals. While he mentions the fact that he has made a study of cat spleens as well as those of many other animals, he neglects in his text to comment particularly on their structure. It may have been that the smallness of the venous system in the cat made it unattractive for detailed study. This however has been a feature which has enabled me to find the solution of the beginnings of this system.

Because the veins in the cat have been shown to have definite beginnings in the pulp spaces does not prove that such a condition exists in other mammals. In the human, sheep, ox, rabbit, guinea pig and dog spleens one finds a very extensive system of venous cap-

illaries which, particularly in the human, almost overshadow that of the pulp. In some animals, as Mollier has shown, the veins consist merely of "retiform spaces following upon one another and reduced to the same calibre to form connected net-like tubes with perforated walls." In others, particularly in the human spleen, they have well defined walls made up of a protoplasmic syncytial lattice of longitudinal bands united by transverse bridges and supported by a reticulum; a perforated structure having stomata of various sizes and shapes. The stomata however are so numerous and of such size that one can readily conceive of an adequate flow of blood elements through them from the pulp to the venous system without the necessity for any definite beginning to the system. On the other hand, in the cat the venous capillaries are short and for the most part bounded by a protoplasmic lining with very few stomata. Such a system must have an adequate number and size of openings to allow for a sufficient flow of blood from the pulp to veins when the necessity arises. These I think I have demonstrated quite conclusively in the large stomata of the primordial branches which have their beginnings *de novo* in the pulp.

Summary

1. The venous capillary system in the spleen of the cat has been shown to have definite points of origin in the pulp.

2. The venous capillary system in the spleen of the cat is independent of the arterial system.

3. The independence of the venous capillary system as seen in the cat spleen may be taken as further proof of the open circulation of the spleen.

Note: I am indebted to Professor Klotz for much helpful advice and guidance in the conduct of this work.

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DESCRIPTION OF PLATES

NOTE: The illustrations may be viewed stereoscopically by placing the narrow edge of a blotter midway between the two pictures, the nose touching the opposite edge. Two pictures are seen at first but if one continues to focus the eyes on them they will blend into one picture giving a three-dimension view.

PLATE 7

FIG. 1. A primordial vein of the cat's spleen, illustrating its indefinite point of origin in the pulp. Note the large stomata in its first part. × 400.

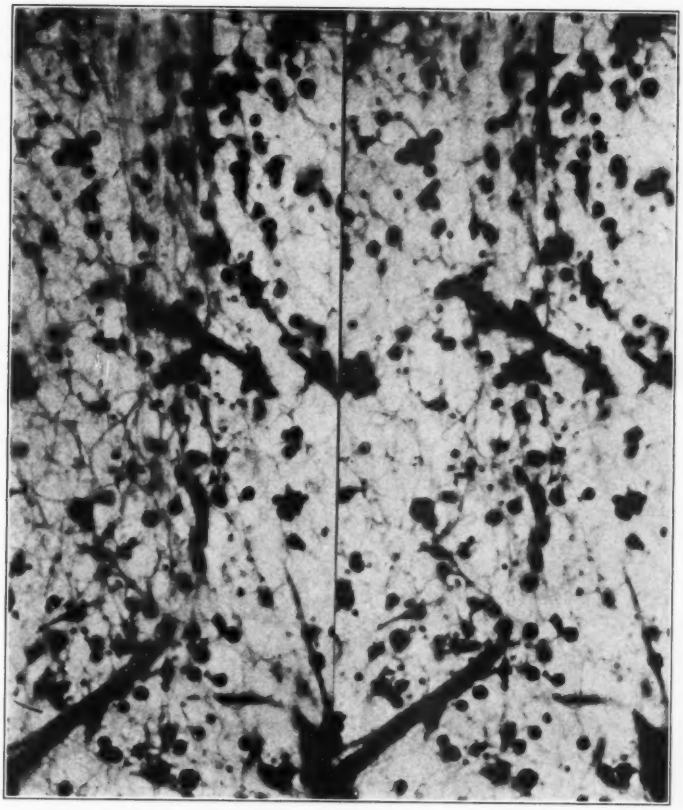

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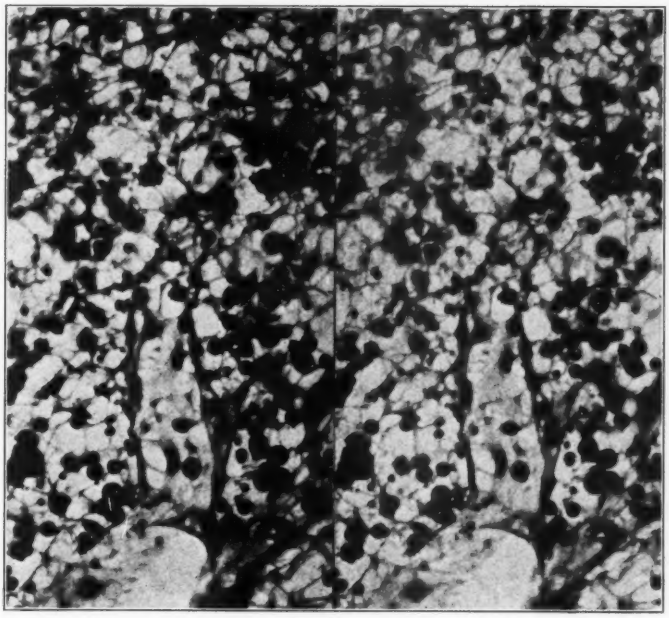
PLATE 8

FIG. 2. A short primordial branch beginning in an ampulla-like dilatation and showing numerous large stomata in its wall. × 400.

FIG. 3. A primordial branch having a more or less definite point of origin in the pulp. From here the walls gradually expand to the full diameter of the vein. Numerous large stomata are seen in its wall. × 400.



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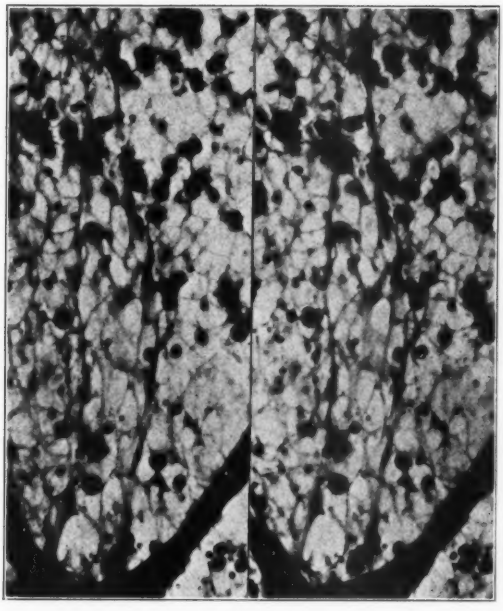

3

Robinson . Venous Drainage of Cat Spleen

NEURO-EPITHELIOMA (GLIOMA) OF RETINA WITH METASTASES *

C. H. Hu, M.D.

(From the Department of Pathology, Peking Union Medical College, Peiping, China)

Cases of neuro-epithelioma (glioma) of the retina with metastases to organs are rather rare. In the following two cases a striking picture of metastases was found at the postmortem examination. The findings in the first case are especially valuable, since it was possible to carry on the autopsy to its utmost completeness.

CASE 1. *Clinical History:* A Chinese boy, 4 years old, came to Peking Union Medical College Hospital April 30, 1925, because of a protruding mass in the left eye. His family history was negative. Two or three years earlier he had had smallpox. Following the attack of smallpox a scar was seen in the cornea of the left eye which was at that time found to be "blind." Three and a half months before admission a tumor appeared in the left eye, accompanied by local discomfort. This tumor grew rapidly, reaching the size of a man's fist at the time of admission. Physical examination showed the child to be underdeveloped and undernourished, with the left eye completely destroyed by a large protruding tumor mass (Fig. 1). The right eye was normal. On the head were three small masses; one in the left frontal region, another in the left temporal region, and the third in the left preauricular region. Cervical lymph nodes were moderately enlarged. On May 1, 1925 the tumor of the left eye was removed by exenteration of the orbit.

Pathological Report

Specimen is that of a tumor of the left eye, soft in consistency, red or dark red in color and measuring 8 by 6.5 by 6 cm. The eyeball is not recognizable. A portion of the skin of the upper eyelid is attached to the tumor. The surface of the extra-orbital portion of the tumor is rough and covered with fibrinopurulent exudate. On section the cut surface is generally soft and dark red with a few scattered areas of yellow, but in the center there is one grayish white area measuring about 2.5 by 1 cm.

Microscopic Examination: Microscopically, under low magnification, the tumor appears very cellular with only a small amount of fibrous stroma. In the tumor tissue there are many areas of necrosis

* Received for publication August 16, 1929.

and hemorrhage. The necrosis involves especially the cells which are a short distance away from the blood vessels. Under high magnification, two types of tumor cells are seen, both showing numerous mitotic figures. The first type (Fig. 15), to which the majority of the cells of the tumor belong, are small, rounded and lymphocyte-like with only a small amount of cytoplasm, and with nuclei which are round and deep-staining. The other type (Figs. 10, 11, 12, 13 and 14) consists of elongated or columnar epithelial-like cells arranged as a rule in single rows. Their nuclei are oval or elongated and at the poles of each there is found a moderate amount of cytoplasm. The cytoplasm at the base of the cell sometimes stretches out to form a pseudopod of varying thickness, which is attached to a thin band of fibrous tissue or to the wall of a blood vessel. The cytoplasm at the opposite end of the cell shows only slight irregularity of the free surface caused by the presence of small protoplasmic excrescences. The free surface of these cells is frequently covered with either round or necrotic cells, or is in close approximation with the free surface of the similar epithelial-like cells which are arranged in the opposite direction. These cells frequently tend to form curved rows or rosettes. Those which form the rosettes usually have no pseudopods nor fibrous nor vascular attachments. Each rosette consists of from ten to thirty or more cells. The lumina of many of the rosettes contain loose cells, both living and necrotic. No typical rods and cones are found. Neuroglia fibers are not demonstrated by the phosphotungstic acid hematoxylin stain.

Diagnosis: Neuro-epithelioma (glioma) of retina with extrabulbar extension.

On May 3, 1925, a fluctuating swelling, 3.4 by 2.2 by 0.5 cm., appeared in the right superciliary region. Following this, numerous other swellings appeared. A photograph of the patient on June 6, 1925, is shown in Figure 2. On June 25, 1925, the patient died.

Autopsy Report

The body is that of a well developed but greatly emaciated Chinese boy weighing 7285 gm. and measuring 88 cm. in length. The left eye is absent with the optic cavity partly filled with soft, gray, necrotic tissue. The right eye is pushed downward by a tumor mass 6 cm. in diameter which extends up into the right superciliary region. The cornea shows a small ulcer in the center. The lower lid is

swollen. Over the left frontal eminence is a tumor mass 5 cm. in diameter. Another mass the size of a walnut is in the left preauricular region. A few others are palpable underneath the scalp along the parietal and the parietofrontal sutures. Only the cervical and the inguinal lymph nodes are palpable, some of these being about 2 cm. in diameter. The left parotid gland is large and infiltrated by tumor tissue.

Peritoneal Cavity: The peritoneal cavity is normal except that the retroperitoneal lymph nodes are greatly enlarged, some of them along the pelvic brim reaching 4 cm. in diameter (Fig. 9). They are filled with white soft tumor tissue which on section shows areas of necrosis and hemorrhage.

Pleural Cavities: On each side of the spinal column and on the inner surfaces of the ribs are several white or hemorrhagic, soft tumor masses either rounded or irregular, each well covered by the parietal pleura. The largest mass is 5 cm. in diameter.

Skull and Dura: On separating the scalp from the calvarium large, soft, hemorrhagic tumor masses are exposed. The largest measuring 5 cm. in diameter is in the frontal bone and the others which are smaller are found along the cranial sutures (Figs. 3 and 4). These masses have worn through the calvarium and are deposited on the external surface of the dura (Fig. 5). The inner surface of the dura shows only two rounded tumor masses, each measuring about 2 cm. in diameter, and projecting into the left frontal lobe of the brain. When the dura was removed these two masses were left buried in the brain. The superior longitudinal, the left lateral, and the left superior and inferior petrosal sinuses are filled with thrombi.

Brain: (Fig. 6). Large and small areas of hemorrhage are found on the surfaces of the left frontal lobe and the anterior portion of the right parietal lobe. The superficial cerebral veins in these hemorrhagic areas are mostly filled with firm, grayish white, inelastic thrombi. The two tumor masses which broke away from the dura when the latter was removed are found in the left frontal lobe, one situated in the posterior portion of the superior frontal gyrus, and the other in the anterior portion of the middle frontal gyrus. The brain tissue in the immediate vicinity of these tumor masses is greatly compressed and shows multiple hemorrhages. Sections made through the entire brain show wide-scattered hemorrhages in the fissures and in the adjoining brain substance. The hemorrhage is especially marked

in the left temporal lobe, in which the brain substance has been converted into a soft, dark red mass. The right optic nerve is normal in gross. The entire left optic nerve, except for 1 mm. near the optic chiasm, is infiltrated with tumor and the extracranial portion of the nerve is necrotic.

Spinal Cord: The spinal cord is normal. In the upper sacral region a few small masses of soft white tumor tissue are present outside of the dura and in the pia-arachnoid.

Bones: The tumor masses in the calvarium have already been described. In the head the tumor tissue is also found in the body of the sphenoid bone, the sinus of the frontal bone, the basilar process of the occipital bone, the bones of the left orbit and the mandible. In the latter the tumor masses are found as subperiosteal swellings on the external surface of the left ramus and on the external and internal surfaces of the right ramus. Longitudinal sections of all the bodies of the vertebrae show on the cut surface large and small areas of tumor tissue, irregular, opaque and yellowish white, in marked contrast to the red color of the surrounding myeloid tissue (Fig. 9). Similar opaque, yellowish white or gray areas are also found in the following bones: humeri (Fig. 8), scapulae, ribs, sternum, pelvic bones, femurs (Fig. 7) and calcanei. No evidence of tumor is found in the other bones. The tumor in the scapulae and the ilia has lifted up and worn through the periosteum, and extended into the adjoining muscles (the supraspinatus and the infraspinatus, the subscapular, the gluteus medius, and the iliopsoas). In one humerus, and in both femurs the tumor has also worn through the cortex, forming subperiosteal swellings. All other organs are normal.

Microscopic Examination: The metastatic tumors in different localities (Figs. 16, 17, 18 and 19) show histological characteristics essentially similar to those of the primary tumor except that no rosettes are found, although there is a general tendency for the tumor cells to become elongated, especially in the bone marrow. The perivascular arrangement of the tumor cells, the necrosis and the hemorrhage are very conspicuous in some places. The amount of stroma is small, and in many places is represented by only a few delicate fibers radiating from the wall of the blood vessels of the tumor.

Anatomical Diagnoses: Neuro-epithelioma (glioma) of retina with metastases to eyelids, left optic nerve, dura, meninges of spinal cord;

bones (frontal, parietal, occipital, sphenoid, bones of the orbit, mandible, scapulae, ribs, sternum, humeri, femurs, calcanei, bodies of vertebrae); muscles (supraspinatus, infraspinatus, subscapulae, gluteus medius, iliopsoas); lymph nodes (cervical, retroperitoneal, inguinal); pleura, and parotid gland; and thrombosis of cerebral veins with extensive hemorrhage in the brain.

CASE 2. *Clinical History:* A German boy, 3 years old, was admitted to Peking Union Medical College Hospital on Nov. 11, 1921 because of protrusion of the left eyeball for eighteen months. The family and past histories were unimportant. Eighteen months earlier his parents noticed a very faint yellowish spot in the pupil of his left eye which was then found to be blind. Six or seven months before admission this eye became more protruding. Frequent vomiting and nausea started three weeks ago. Physical examination showed the left eyeball to be conical in shape and protruding (Fig. 10), the anterior chamber shallow, the pupil round, widely dilated, no reaction to light, lens opaque and dislocated upward and to the temporal side. Operation for the exenteration of the orbit was done on Nov. 12, 1921. Toward the end of the operation patient stopped breathing and died.

Pathological Report

Eye slightly enlarged. Horizontal diameter 24.5 mm., anterior-posterior diameter 27 mm. Cornea clear and conical. Pupil dilated, immobile and slightly eccentric. Anterior chamber shallow. Posterior sclera invaded by pinkish gray tumor tissue. On equatorial section a tumor is found filling up the vitreous space and firmly attached to the retina and choroid on one side near the equator. Posteriorly the tumor covers the retina and the optic disc; anteriorly it covers the lens and the ciliary body. Behind the retina there is a layer of tumor tissue 1.5 mm. thick. The optic nerve is thick, friable and infiltrated by tumor tissue which is also present in the tissue removed from the optic cavity.

Microscopic Examination: Microscopically the tumor is found to be very cellular, consisting of closely packed cells with rounded or oval nuclei and a very small amount of cytoplasm. There are many mitotic figures (Fig. 22). No rosettes are found. The cells are well preserved around the blood vessels which are present in large numbers, but those not in the immediate vicinity of the vessels are necrotic (Fig. 21). The retina is entirely destroyed by the tumor in the region about the optic disc, but farther away it is still intact. The vitreous is largely filled with tumor. The choroid is destroyed in places and its branching pigmented cells may be found scattered in

the thick layer of tumor tissue between the pigmented epithelium and the sclera. The sclera is intact, although thinned out in places. No point is found at which the tumor definitely penetrates the sclera. The tumor is present, however, outside of the sclera, enclosing the whole posterior aspect of the eyeball. The optic disc and the optic nerve are infiltrated by tumor and their normal structures are no longer recognizable. The tissue removed from the optic cavity also shows tumor.

Diagnosis: Glioma of retina of the left eye with extension into the left orbit.

Autopsy Report

The body is that of an emaciated white boy, weighing 9 Kg., and measuring 84 cm. in length. The left orbit is exenterated. The right eye is normal. None of the organs is remarkable except for the following condition noted in the central nervous system: The surface of the cerebral cortex is covered with many minute grayish islands of tumor tissue in the pia-arachnoid, more marked along the vessels in the sulci than over the surface of the convolutions. At the base of the brain and on the surface of the cerebellum, pons and medulla, these islands coalesce and form larger and thicker patches varying from a few millimeters to 3 or 4 cm. in diameter (Fig. 23). Anterior to the optic chiasm is a soft, gray, oval tumor mass about 1.6 cm. in length, and 1 cm. in width and in thickness, pressing upon both the left and right optic nerves and on the chiasm itself. On section the superficial portions of the cortex of cerebrum and cerebellum are found to be invaded by small nodules of tumor from the meninges. The lateral ventricles are slightly dilated. The ependymal surface is covered by numerous rounded or flattened, single or conglomerate tumor nodules measuring up to 5 mm. in diameter. Larger tumor masses are found in the choroid plexus. The intra-orbital portion of the right optic nerve is infiltrated with tumor. The spinal cord, especially its lower half, is greatly thickened and fills the dural cavity tightly. Its pia-arachnoid is extensively infiltrated by soft, grayish tumor tissue. On section the cut surface shows the tumor in the meninges invading the cord from all sides, especially from the dorsal side. A few small tumor masses are also found in the cauda equina.

Microscopic Examination: The tumors in the brain and spinal cord (Figs. 24, 25, 26, 27 and 28) show the same type of cells as those

of the primary tumor in the left eye. Most of the secondary growth is found in the pia-arachnoid, covering the larger part of the surface of the brain and the cord. In some places it extends directly into the brain or the cord from the surface. In others it fills up the Virchow-Robin's spaces and travels with the blood vessels into the deeper nervous tissue. The choroid plexus is almost entirely replaced by the tumor. The spinal nerve roots are either completely surrounded by or heavily infiltrated with tumor. There is no evidence of tumor in the right eye, but the right optic nerve is found to be completely surrounded by a layer of tumor tissue.

Anatomical Diagnoses: Glioma of retina of left eye with extension to the left orbit, brain, choroid plexus, spinal cord, meninges and the right optic nerve.

Discussion

Tumors of the retina, as illustrated by the above two cases, have long been called gliomas. It is, however, generally recognized that the glioma of the retina is quite different from the ordinary glioma of the brain in its rapid growth, greater tendency toward metastasis and its lack of glia fibers demonstrable by ordinary staining methods. In 1891 Flexner [1] first showed the external granular layer of the retina as the origin of the tumor and called attention to the fact that cells of the "rosettes" corresponded to the rod cells and cone cells of the retina. He therefore proposed the name neuro-epithelioma of retina, which was adopted by Wintersteiner [2] in 1897. Lately, however, the term retinocytoma has been suggested by Mawas [3] on account of the close resemblance of the neoplastic cells to the undifferentiated cells of the embryological retina. On the other hand, Urra [4] and Ascunce,[5] having succeeded in demonstrating the presence of glia fibers in this type of tumor, are in favor of calling it by its previous name — glioma. Finally, Bailey and Cushing,[6] who are inclined to believe that the cells of the rosettes are primitive spongioblasts, have named the tumor containing rosettes spongioblastoma primitivum retinae and that containing neuroglia cells or nerve cells (both of which are derived from the retinoblasts) retinoblastoma.

This wide variation in terminology is largely due to the fact that this type of tumor is essentially an embryonal multipotent new-growth. While its cells generally are not sufficiently differentiated to give one any definite information as to its true nature, it may in

various cases produce neuroglia cells, rod cells and cone cells, or even nerve cells.[7] This fact at once forces one to regard the retinal epithelium as the origin of the tumor, for it is only from this structure that all these elements can develop. The embryonal character of this type of tumor is further illustrated by the morphological resemblance of the tumor cells to the cells of the embryonal retina, as already pointed out by Collins [8] and Mawas, by its rapid growth, and by its occurrence chiefly in young children.

If this type of tumor is recognized as originating from the embryonal retina, it then becomes a simple matter to account for its microscopic variations — the presence of the undifferentiated cells, the difficulty with which the glia fibers are demonstrated by ordinary methods, and the inconstant presence of the neuroglia, neuroepithelial or nerve cells. The presence of rosettes in the primary tumor of Case 1 and their absence in the metastases can then be explained by the more complete differentiation of the cells of the primary tumor than of those of the metastatic growths.

It also becomes apparent that although we are still employing such terms as neuro-epithelioma or glioma of the retina, they are inadequate to designate this type of tumor, for such terms express the unipotent nature of the tumor which is, as a matter of fact, multipotent.

Since the retinal epithelium is formed by the budding off of the medullary epithelium which later develops into the central nervous system, we may expect to find tumors of the brain and the spinal cord which are analogous to those found in the retina. This is indeed the case. In the so-called neuro-epithelioma of the brain, structures similar to the rosettes of the retinal tumors are found. In the tumors called medulloblastoma by Bailey and Cushing both neuroglia cells and neuroblasts may be identified. These tumors, furthermore, present the same embryonal characteristics of the retinal tumor in their rapid growth, lack of cell differentiation, inconstant presence of one or another element and tendency toward spreading into the meninges.

In regard to metastasis Wintersteiner gives the frequency with which the various organs are involved as follows:

TABLE I

Brain and meninges	43 times
Skull and bones of face	40 "
Neighboring lymph nodes	36 "
Parotid	9 "
Skeletal bones	9 "
Liver	7 "
Spinal cord and meninges	5 "
Kidneys	2 "
Ovaries	2 "
Lungs	1 time
Spleen	1 "

To the above table the following cases of metastases to the distant organs may be added from the literature:

TABLE II

Brain and meninges	2	(Knapp,[9] Taylor and Fleming [10])
Skull and bones of face	3	(Keys,[11] Knapp,[9] Taylor and Fleming [10])
Distant lymph nodes	2	(Fehr,[12] Taylor and Fleming [10])
Skeletal bones	4	(Fehr,[12] Gardiner,[13] Knapp,[9] Taylor and Fleming[10])
Liver	2	(Knapp,[9] Radcliffe and Goldberg [14])
Lungs	1	(Fieber [15])
Testes	1	(Gardiner [13])

From the above tables it will be seen that metastases in distant organs by way of the blood stream and the lymphatic stream are not common, as there are only twelve cases in which the skeletal bones are involved as in Case 1. On the other hand, secondary growths in the head are much more frequent and this is generally attributed to the direct extension of the tumor. This is certainly true in Case 2 and probably so to a certain extent in Case 1 in which the head is especially involved. In Case 2 the spreading of the tumor by direct extension is as definite as it is remarkable. Starting from the eyeball, the tumor at once becomes widespread without breaking the continuity in the entire central nervous system. Its distribution reminds one of that of the cellular exudate in cases of acute meningitis, or that of the fluid material artificially injected into the meningeal spaces. This mode of spreading is possible, however, only when the tumor is rapidly growing and when the stroma is insufficient to keep the loose tumor cells from spreading readily into places offering the least resistance.

Attention also may be called to the fact that from the above tables there are only five cases recorded in which the spinal cord and its meninges are involved as in Cases 1 and 2. In view of the frequent involvement of the brain, the small number of cases of cord involvement reported is probably due to the fact that the cord is not always examined at the time of autopsy.

Summary

Two cases of tumor of the eye, commonly known as glioma or neuro-epithelioma of the retina, are reported: one with metastases to the skull, skeletal bones, muscles, lymph nodes and meninges; and the other with extension to brain, spinal cord and meninges. The embryonal character of these tumors and their analogy to certain tumors of the central nervous system are stressed.

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DESCRIPTION OF PLATES

PLATE 9

FIG. 1. Case 1. Photograph of the patient at the time of admission. A slight elevation under the skin in the left superciliary region is indicated by an arrow.

FIG. 2. Case 1. Photograph of the same patient taken forty-two days later. The primary tumor had been removed: the subcutaneous swellings are prominent.

FIG. 3. Case 1. The external surface of the calvarium showing a large metastatic tumor mass over the left frontal prominence and smaller masses along the cranial sutures.

FIG. 4. Case 1. The internal surface of the calvarium showing metastatic tumor masses. The ragged appearance of the tumor is caused by the separation of the calvarium from the dura.

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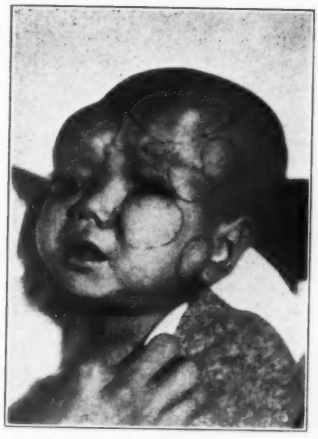

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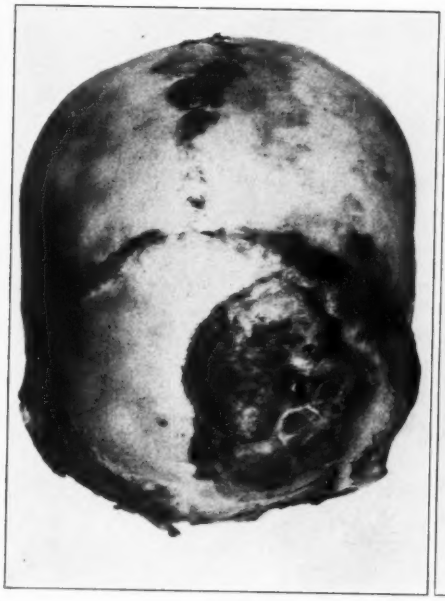

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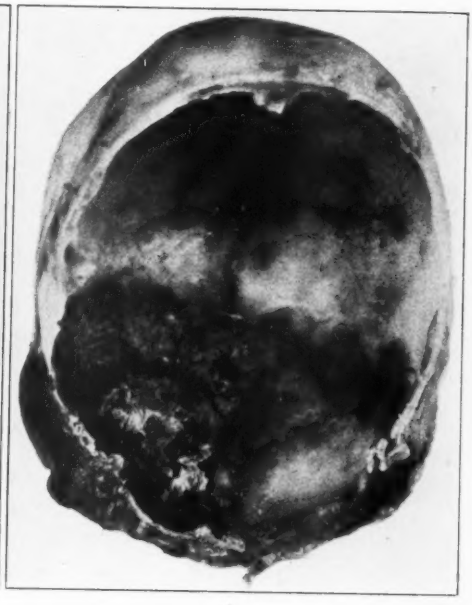

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Hu

Neuro-epithelioma (Glioma) of Retina

Plate 10

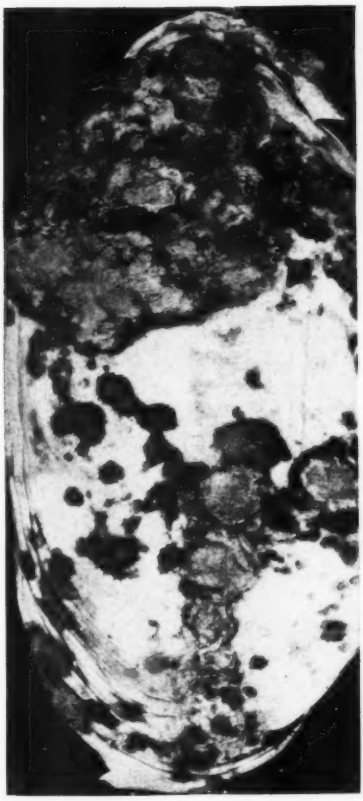
Fig. 5. Case 1. The external surface of the dura showing one large and many small masses of metastatic tumor.

Fig. 6. Case 1. Brain, frontal view, showing extensive hemorrhage and two tumor masses in the left frontal lobe. Most of the cerebral veins in the hemorrhagic areas are thrombosed.

Fig. 7. Case 1. Left femur, showing tumor metastasis filling up most of the medullary cavity. Two masses of the newgrowth are found under the periosteum. The cortex near the neck of the femur has been worn through by the tumor.

Fig. 8. Case 1. Right humerus showing metastatic tumor in the medullary cavity.

Fig. 9. Case 1. Longitudinal section of the spinal column showing tumor metastases in the bodies of all the vertebrae. A few rounded tumor masses can also be seen in the pelvis.



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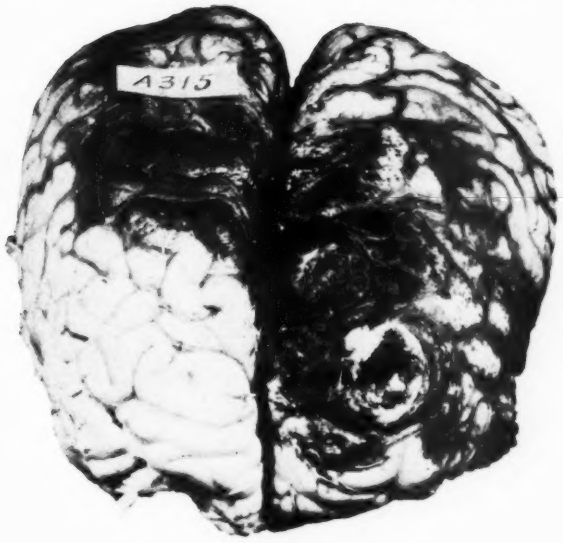

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Neuro-epithelioma (Glioma) of Retina

Plate 11

Fig. 10. Case 1. Primary tumor of the left eye showing rosettes. × 100.

Fig. 11. Case 1. Primary tumor of the left eye showing rows of straight and curved epithelial-like, columnar cells. × 250.

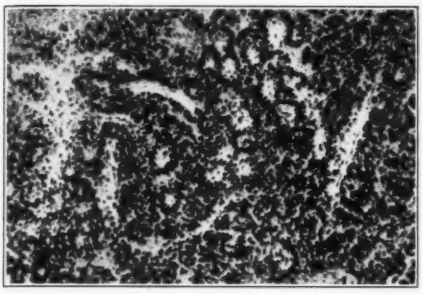
Fig. 12. Case 1. Primary tumor of the left eye showing a row of columnar cells attached to a small band of connective tissue and covered on its surface by a few necrotic cells. × 500.

Fig. 13. Case 1. Primary tumor of the left eye showing rosettes. × 500.

Fig. 14. Case 1. Primary tumor of the left eye showing a curved row of epithelial-like columnar cells with sharp cell borders on one side. × 500.

Fig. 15. Case 1. Primary tumor of the left eye showing invasion of the intraocular muscle. × 100.

Fig. 16. Case 1. Metastatic tumor in dura showing the tumor cells better preserved around the blood vessels than those away from them, resulting in what appears to be a perivascular growth. × 100.



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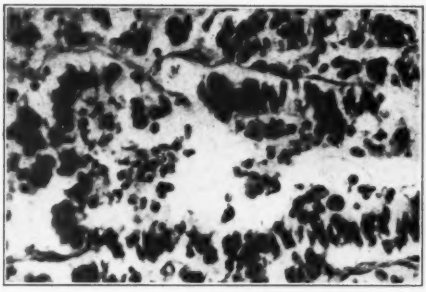

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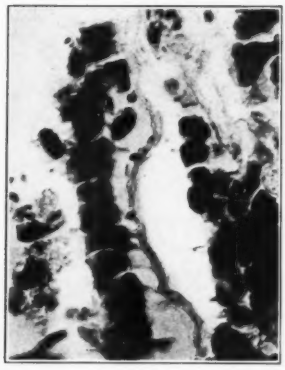

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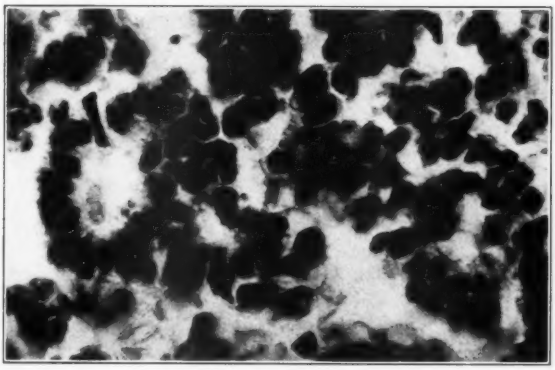

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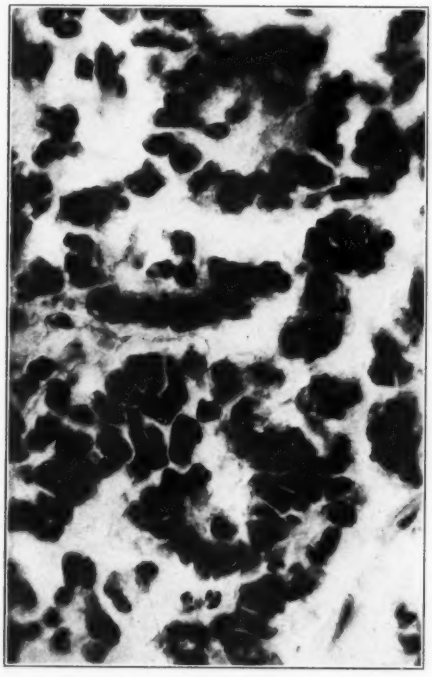

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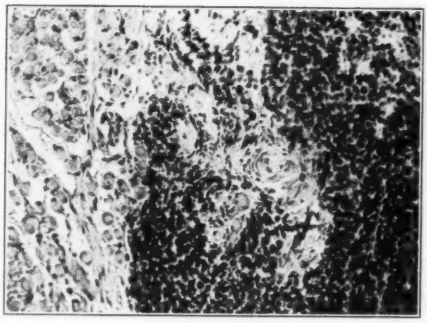

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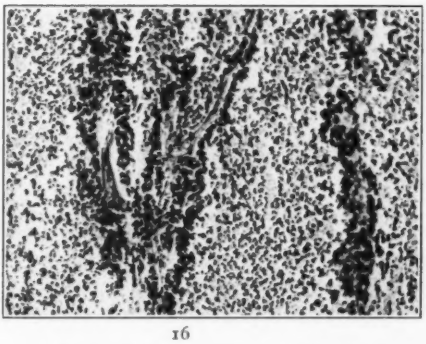

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Neuro-epithelioma (Glioma) of Retina

Plate 12

Fig. 17. Case 1. Tumor metastasis in the bone marrow of rib. × 100.

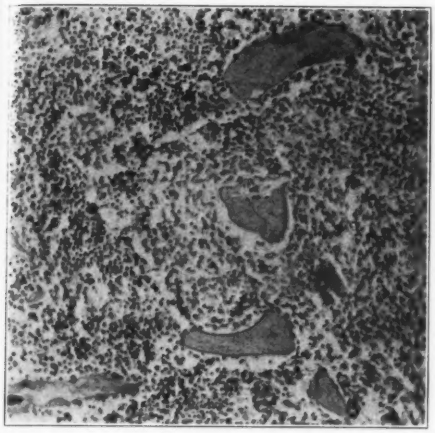
Fig. 18. Case 1. Tumor metastasis in the bone marrow of rib, showing two mitotic figures. × 1000.

Fig. 19. Case 1. Meninges of spinal cord, showing tumor cells. × 100.

Fig. 20. Case 2. Photograph of the patient at the time of admission.

Fig. 21. Case 2. Primary tumor of the left eye, showing the apparent perivascular grouping of the tumor cells following necrosis of those cells farther away from the blood vessels. × 60.

Fig. 22. Case 2. Primary tumor of left eye, showing two mitotic figures. × 800.



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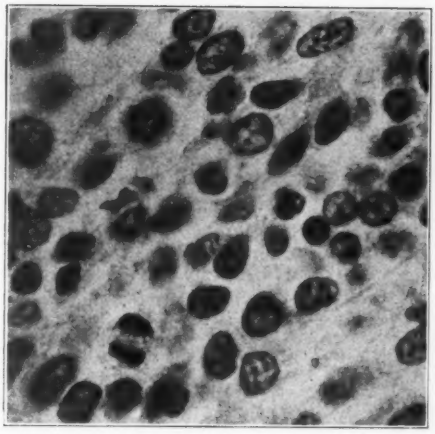

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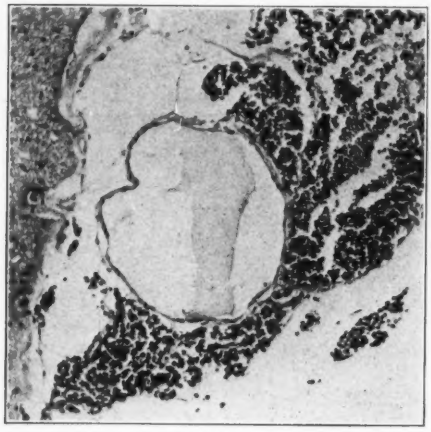

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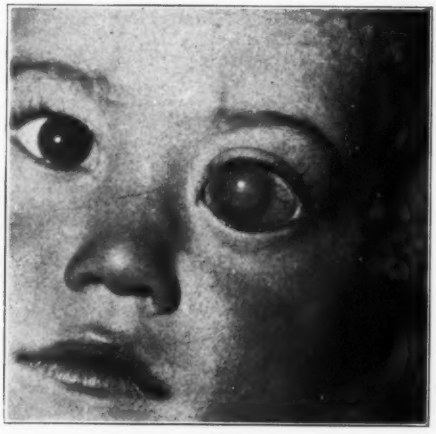

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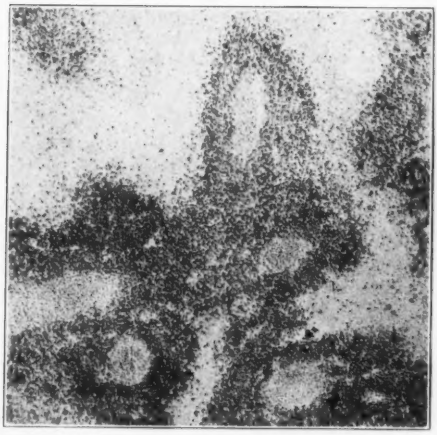

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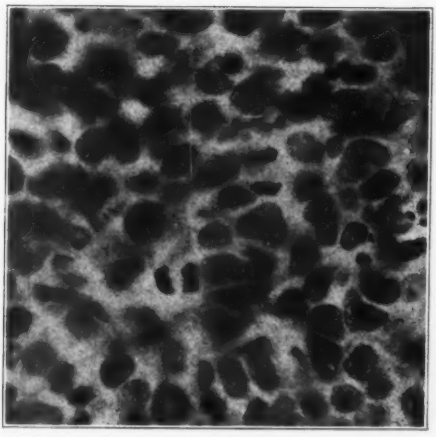

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Hu

Neuro-epithelioma (Glioma) of Retina

PLATE 13

FIG. 23. Case 2. Gross appearance of cerebellum with metastatic tumor growth on its surface.

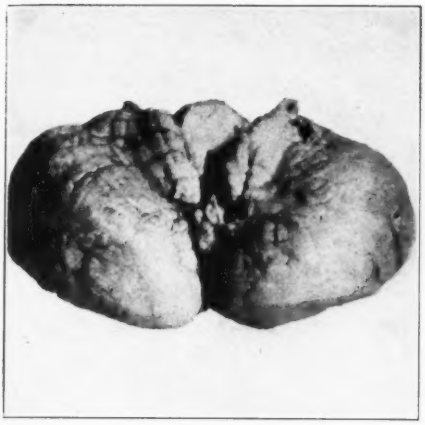
FIG. 24. Case 2. Cerebellum, showing metastatic tumor infiltrating the meninges and invading the cerebellar cortex. × 20.

FIG. 25. Case 2. Metastatic tumor in the meninges of cerebellum. × 600.

FIG. 26. Case 2. Spinal cord, thoracic portion, showing extensive infiltration of the meninges and invasion of the cord by tumor. Sections of cord at different levels show essentially the same picture.

FIG. 27. Case 2. Spinal cord showing infiltration of tumor cells in the Virchow-Robin's spaces of the blood vessels in the white matter near the anterior horn. × 80.

FIG. 28. Case 2. Anterior nerve roots of the spinal cord surrounded by and infiltrated with tumor cells. × 60.



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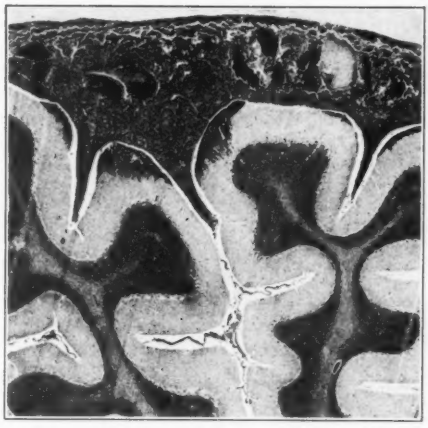

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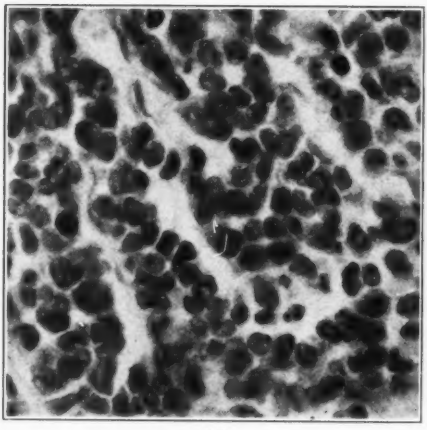

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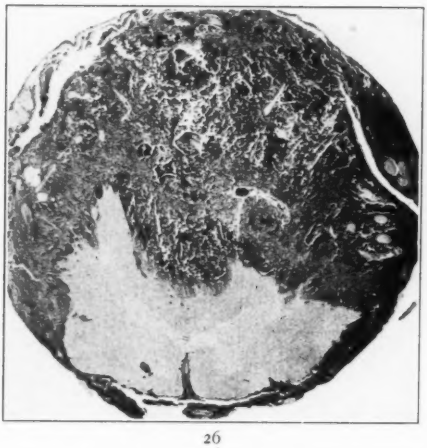

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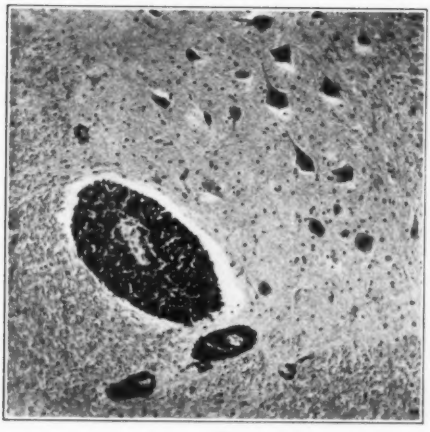

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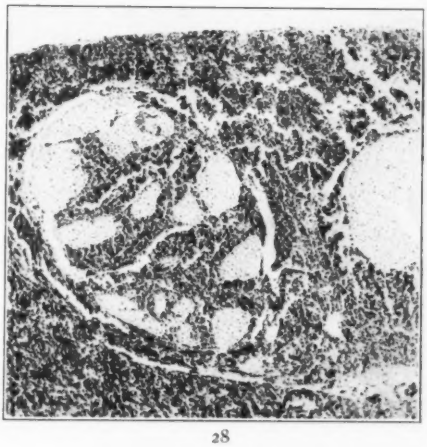

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Neuro-epithelioma (Glioma) of Retina

ORIGIN OF THE PERIVASCULAR PHAGOCYTES OF GRANULATION TISSUE *

F. A. McJunkin

(From the Department of Pathology, Loyola University School of Medicine, Chicago, Ill.)

That ameboid mononuclear phagocytes may arise from the vascular endothelium during early embryonic development has been established.[1, 2] Whether phagocytes detach themselves from the vascular endothelium of the adult is controversial. An extensive literature dealing with the problem has accumulated. The vital reaction of cells to dyes and particulate matter, supravital staining methods and tissue culture have been the chief technical methods employed in the investigations of the last few years. As a result of the facts brought out by these newer methods the idea is now prevalent that a majority of the blood and tissue mononuclear phagocytes are derived from the reticular or lymphoid tissue. The cells of this origin are now commonly called monocytes. This view, in my opinion, is correct and it greatly clarifies our conception of the mononuclear phagocytes. Much difference of opinion still exists in regard to the relationship and distribution of the parent reticular and lymphoid tissues. Maximow's [3] extensive investigations led him to think that the lymphocytes themselves were transformed into mononuclear phagocytes (polyblasts). Sabin, Doan and Cunningham [4] by supravital staining with neutral red demonstrated within the monocytic cytoplasm a rosette of dye granules, and they produced evidence that this monocyte is of reticular origin. These authors did not, however, examine in detail the distribution of the reticular tissue in the body. Both Maximow and Sabin and co-authors were of the opinion that the monocyte did not comprise the entire group of mononuclear phagocytes. The latter recognized a second type of cell, the clasmatocyte, which is derived from the vascular endothelium. Maximow on the other hand is emphatic in his denial that the vascular endothelium of the adult contributes to the group of mononuclear phagocytes, but he expressed the opinion that certain primitive or embryonic cells persist in the extravascular tissues as "resting wan-

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dering" cells and they in response to stimulation become active phagocytes. He thought that the cells lining the capillaries of sinusoidal organs such as the spleen, liver and bone marrow were both actively phagocytic and a source of detached phagocytes. However, he did not think that the lining cells of these blood channels were true endothelial cells but he regarded them as histiocytes and unrelated to true endothelium.

Mallory [5] was the first to recognize endothelium as the fixed tissue source of the mononuclear phagocytes and he has observed and clearly stated [6] that the lymphoid reticulo-endothelium plays an important rôle in the genesis of the group of mononuclear phagocytes named by him "endothelial leucocytes." My earlier investigations [7] convinced me that the reticulo-endothelium (lymphatic and blood-vascular) was the chief source of mononuclear phagocytes in the adult. Until recently, I [8] have entertained the possibility that the peroxydase-reacting phagocytes found in normal human blood may arise in the bone marrow, which is the view held by Naegeli. [9] However, monocytes have the property of ingesting peroxydase granules and these granules for a time retain their ability to react with benzidin.[10] If it be true, as these experiments indicate, that the peroxydase mononuclear phagocytes, or monocytes of human blood acquire their granulation secondarily in a more or less accidental fashion then my conclusion is that all mononuclear phagocytes separate from the reticulo-endothelium, either lymphoid or blood-vascular. In my first experiments [7] with carbon suspensions no distinction was made between endothelial leucocytes of lymphoid origin and those derived from the blood-vascular endothelium since both kinds were marked by the carbon in a somewhat similar fashion. Later experiments [11] with the direct supravital staining of peritoneal exudates, of the blood and of various tissues, and with vital reactions on tissue cultures of lymph nodes [12] introduced new evidence that tended to show a division in the group. The response of the reticular tissue of lymph nodes appearing in tissue cultures to vital tests was a convincing demonstration of the origin of monocytes from this tissue. These experiments indicated that there were at least two kinds of phagocytes one of which was derived from lymphoid reticular tissue. It was felt that evidence of the origin of the second type from blood-vascular endothelium was inconclusive. The earlier observations [7] were made on vascular endothelium of the sinusoidal type,

the true endothelial character of which has been disputed. The capillary endothelium has now been made a subject of further study. Tissue cultures proved of little help since this type of endothelium either does not grow readily, or if these cells multiply they are not easy to identify because of course the structures formed carry no blood. Granulation tissue produced experimentally has been employed for the observations. The first step was to show that the true endothelium of the vascular sprouts is phagocytic, since this has been denied. It was found in experiments already published [13, 14] that when stimulated by trypan blue injections this endothelium is fully as active in the ingestion of carbon as is the sinusoidal endothelium of liver and spleen. In the experiments described in this paper the behavior of the endothelium and the cells in contact with or near it have been observed under varying conditions after having been stimulated and manifesting phagocytosis.

Perivascular Phagocytes of Endothelial Sprouts Marked with Carbon

Rabbits and rats were used. The granulation tissue was produced by subcutaneous injections of a saturated alcohol-acetone solution of Sudan III or a temporary suspension of the dye in water. Carbon in the form of India ink was injected intravenously (ear vein or tail vein). A number of minor variations were introduced especially by changing the time between the different procedures. As far as the problem now under consideration is concerned it is necessary to select and describe those experiments in which the essential results were seen to best advantage. The tissues were fixed in Zenker's fluid, embedded in paraffin and stained with hematoxylin-eosin.

Four-Hour Rabbit (*Experiment 26*): Two rabbits weighing 1200 and 1800 gm. each were given 2 cc. Sudan III in acetone-alcohol subcutaneously in each groin. On the same day 5 cc. trypan blue (saturated aqueous solution) were injected intravenously. The trypan blue injection was repeated on the second and fourth days. On the eleventh day 4 cc. of India ink (Higgins') were injected intravenously. The ink was injected slowly under ether and in two doses one hour apart. The animals were killed and the groin tissue fixed four hours after the first ink injection. As usual the distribution of the carbon is very irregular with large areas in which the capillaries are practically devoid of ink and others where much carbon is present.

No doubt the ink precipitates in the form of loose masses readily separable, and here and there these enter the arteries to a microscopic area. Such areas were selected for study. Again the structure of the capillaries and small vessels varies. In general where the vessels are older the carbon is situated not only in the elongated cells next the lumen but also in second layer cells of the same shape (Fig. 7). They show no pseudopodia. In other locations granulation tissue is in the process of active formation with the endothelia large and plump and here and there a mitosis. Some of these new capillary sprouts are like the usual textbook description with the narrow pointed endothelia, but much oftener the endothelial cells are of a size and shape suggesting phagocytes rather than lining cells (Fig. 3). The presence of red corpuscles is extremely important in establishing the endothelial character of the structures. Carbon is present not only in the innermost cells but also in cells of similar structure only partly in contact with the lumen or completely removed to the second layer. These cells in size and shape have the irregularity of the so-called sinusoidal type of endothelium. These cells in the ink-marked areas contain carbon particles (Figs. 1 and 2). In other places the new vessels form a reticular network in which cells connect by their cytoplasmic processes. Such cells no doubt later arrange themselves to form vessels of the usual type. These large irregular cells may abut on the capillary lumen on one side while on the outer they have reticular processes (Fig. 5). Throughout the experiments there was noted a close resemblance between such structures and the growth of reticular tissue in tissue cultures of lymph nodes.[12]

Discussion of the Carbon Experiments: It seems that the carbon enters the cells by phagocytosis and not passively, as claimed by Lang.[15] In the fixed tissue the ink-marked cells are the larger and more irregular ones and not infrequently they are of an appearance identical with detached phagocytes. All stages of separation from the lumina of capillary structure are readily observed (Figs. 4, 5 and 6). Often it is not possible to determine whether the heaping up of endothelium is toward the lumen or outward, but the evidence is strong that leucocytes do not migrate in to assume these various positions in relationship to the lumina of the vessels. It was rather often seen that a group of ink-containing phagocytes were in the vicinity of a vessel with much carbon in its lumen (Figs. 4 and 6). It is not assumed that all such free cells are detached endothelia. If guinea

pigs are injected intraperitoneally with huge doses of India ink the carbon particles within five minutes appear in the sinuses of the substernal lymph nodes. In this experiment the particulate matter unquestionably passes through cell membranes, with phagocytosis playing no part. Such extravascular carbon may of course be taken up by phagocytes already in the tissue spaces, and so the distribution of carbon-marked cells seen in Figures 4 and 6 would be explained. However, the maximum amounts of intracellular carbon in the ink-marked foci are seen where the endothelia are large and often where they are "heaped up" into more than one layer. This it seems should be considered in connection with the structural and functional identity of the endothelia and the detached phagocytes near the lining cells.

Perivascular Phagocytes of Endothelial Sprouts Marked with Trypan Blue

Full-grown rats were used because they withstood larger doses of trypan blue than did rabbits. The granulation tissue was produced by injection into the groin of 1.5 cc. of an arsenious acid solution made by diluting 0.5 cc. of a saturated aqueous solution of arsenious acid to 6 cc. with 50 per cent ethyl alcohol. Seven days later 2 cc. of a saturated aqueous solution of trypan blue were injected into a tail vein. On the following day 2 cc. of the dye were again injected. The rats were killed two hours, five hours and eighteen hours after the last injection. Inflammatory tissue at the site of the arsenious acid injection shows extensive formation of new capillaries and an inflammatory exudate in which large mononuclear phagocytes predominate. The tissue was fixed in formalin, cut thin for embedding in paraffin after thirty minutes in two changes of acetone and one hour in benzol. After removal of the paraffin from the sections they were mounted in balsam and examined unstained. In this way most of the trypan blue is preserved in the sections which are thin and much better than frozen sections for accurate observation. As a check on the structures, alternate sections have been stained in the usual way with hematoxylin-eosin. In both the five and eighteen-hour animals the dye is sufficiently collected to appear as minute granules in the cytoplasm of both endothelia and detached phagocytes. In the eighteen-hour rats the granules are larger. In the unstained preparations often capillary connections with larger vessels

filled with red corpuscles show the endothelium to best advantage since here the identification of the lining cells is certain. In such locations two to a half-dozen large endothelia may appear in the wall of a capillary, and at the end of five hours and eighteen hours the fine trypan blue granules tend to lie at the ends and external to the nuclei. The distribution of the dye granules is essentially the same as that of the carbon shown in the illustrations. In the same tissue embedded in the usual way and stained with hematoxylin-eosin, dye granules appear only in a few of the extravascular phagocytes where the aggregations of dye are coarse. The tissue used for the carbon experiments was also rapidly embedded and examined unstained so as to preserve the trypan blue. In these the trypan blue was present only irregularly here and there in the form of coarse granules in the cells lining the capillaries, except in animals receiving trypan blue about twenty-four hours before the ink injections, in which instance the picture was more complex with much extravascular dye and a staining of the endothelia like that of the eighteen-hour rats. To test the acute effect of the trypan blue three full-grown rats were injected subcutaneously to produce the granulation tissue, and four days later 2 cc. saturated aqueous solution of trypan blue were injected into a tail vein. The rats were killed five minutes, ten minutes and one hour after the trypan blue injections. In the first two the dye is demonstrable only in the lumina of some of the capillaries where there is a more or less diffuse staining of the corpuscles. In the one-hour rat there are scattered extravascular phagocytes containing blue granules. In some of the endothelia there is a tinge of blue but no granules. Evidently the dye is more or less dissolved whether the sections are frozen and floated on water or rapidly embedded in paraffin, unless sufficient time elapses to permit the cells to concentrate the dye into granules.

Observations and Discussion of the Trypan Blue Tissue: Evans and Scott [16] in their study of the macrophage reaction to acid colloid dyes found that the macrophage was the first to respond but that fibroblasts accumulated the dye after chronic administration and finally had a resemblance to the macrophage. In the acute process (up to about two days) the macrophage only contained the dye. It was only after a long time (seventy-five days) that the fibroblast became heavily loaded. By the methods used in my experiments the trypan blue granules become very distinct in endothelia eighteen hours after

the dye administration, although usually the granules are not so large as in the extravascular phagocytes. However, as shown in the illustrations of the carbon distribution where a reticular structure is assumed by the endothelium it often is not possible to be sure whether a given cell is an extracellular phagocyte or definitely part of a capillary. Evans, Bowman and Winternitz [17] found abundant trypan blue in the Kupffer cells of the liver. Here and in other sinusoidal organs some of the capillary endothelium is kept large and active by the normal phagocytic processes in them. In granulation tissue and in most normal tissues the phagocytic property of the endothelium becomes latent unless it is artificially stimulated. In the liver, as in granulation tissue, the evidence now is that some of the perivascular cells are of blood-vascular origin while others are monocytic. Evidence of this in the sinusoidal organs has been presented elsewhere.[11, 12] The capillary endothelium of granulation tissue collects the colloidal dye with a facility equal to that of the Kupffer cells of corresponding size. Since carbon particles may pass through membranes without undergoing phagocytosis the smaller dye particles no doubt do the same. The presence of dye granules in extracellular phagocytes *per se* is not proof of their origin from the vascular endothelium, but the structural resemblance and the positions of the attached and detached cells indicate a relationship between the two.

Summary and Conclusions

1. That the blood-vascular endothelium other than that found in sinusoidal organs has phagocytic properties is confirmed by these experiments. By experimental stimulation the usual type of capillary endothelium assumes the size and shape of the so-called histiocytic endothelium of the sinusoidal organs such as liver, spleen and bone marrow.

2. In the granulation tissue of animals injected with India ink the arrangement of detached phagocytes about the endothelia and the structural resemblance of attached and detached cells are evidence of the identity of the two.

3. The endothelia of the capillaries of granulation tissue segregate trypan blue to form microscopic granules just as the sinusoidal endothelium does. The evidence obtained by the use of colloid dyes, that phagocytes separate from the endothelium of such organs as the

liver and spleen, applies equally to the endothelium of granulation tissue.

4. These experiments indicate that some of the perivascular phagocytes are derived from the vascular endothelium and are not monocytic.

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DESCRIPTION OF PLATES

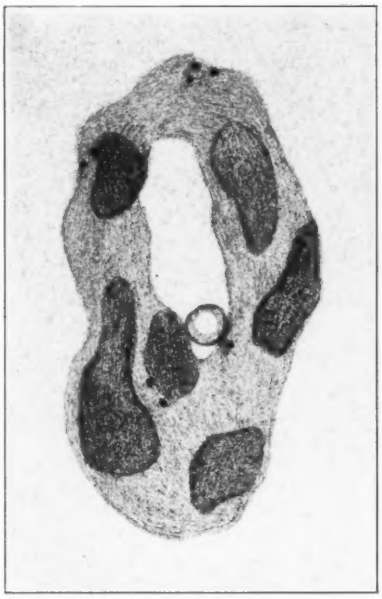
Structures drawn to the same scale with aid of the camera lucida. Tissues from animals receiving intravenous India ink stained with hematoxylin-eosin.

PLATE 14

FIGS. 1 and 2. Two capillaries showing the stimulated endothelium with "second row" cells containing carbon. Some of these have only partial contact with lumina.

FIG. 3. Capillary sprout with the second cell which contains carbon in mitosis. The end cell has the ameboid type of nucleus often seen in the endothelia.

FIG. 4. Much carbon in endothelia and in nearby phagocytes. Often such extravascular carbon within phagocytes is found where the endothelia are large and phagocytic and not where the capillary wall is thin.



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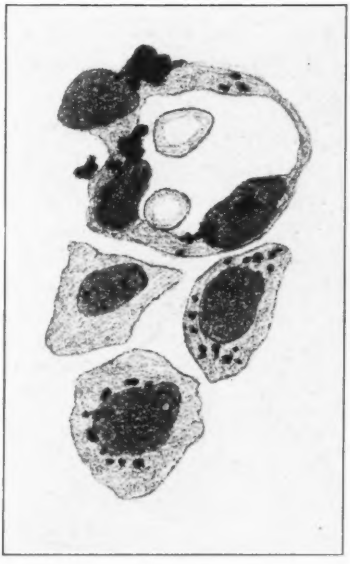

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McJunkin

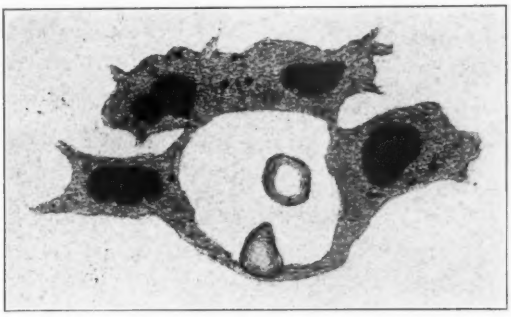
Perivascular Phagocytes of Granulation Tissue

PLATE 15

FIG. 5. Cross-section of capillary where the endothelia assume a reticular appearance. Such structures are common and the proliferating capillaries have this appearance as frequently as that seen in Fig. 3.

FIG. 6. Two capillaries with carbon in endothelia but none in the phagocytes. Monocytes certainly may be of this size and shape. In the capillary at the left the outer carbon-containing cell has the ameboid type of nucleus.

FIG. 7. Longitudinal section of a thin-walled vessel with much carbon in both inner layer and in cells of similar appearance farther out. If the outer cells are of a type different from the endothelia, differentiation is not possible in the sections.



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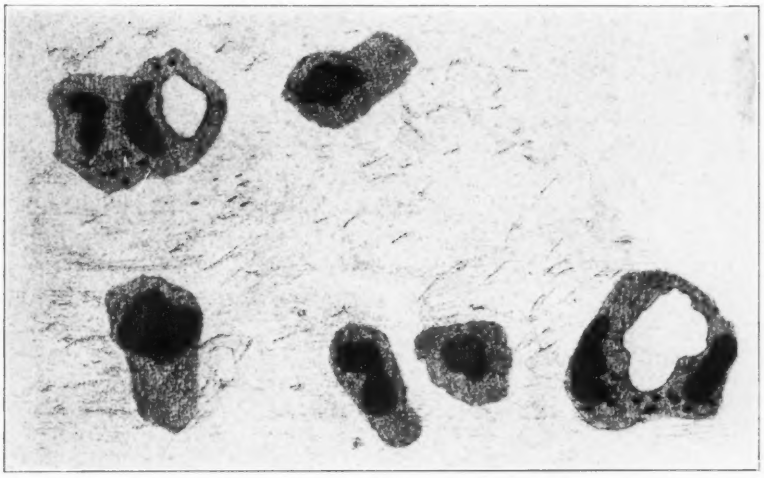

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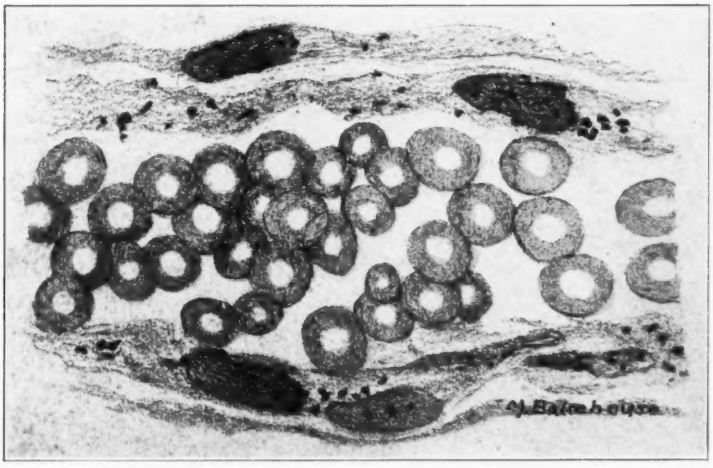

7

METASTATIC INOCULATION OF A MENINGIOMA BY CANCER CELLS FROM A BRONCHIOGENIC CARCINOMA *

B. M. FRIED, M.D.

(From the Surgical Clinic of Dr. Harvey Cushing, Peter Bent Brigham Hospital, Boston, Mass.)

Experimental and clinical investigations on cancer for the last two decades seem to favor the conception that the development of an epithelial malignant disease is largely influenced by immunobiological factors. Apparently not only does the host itself offer resistance to the development of a cancer (natural immunity) but resistance toward the implantation of cancer can be induced experimentally in animals (active immunity). Thus Ehrlich [1] showed that rats with healed mouse cancer are immune to a reinfection, and also that mice with actively growing tumors are mostly immune to secondary implants. Similarly Murray [2] reported that mice with a tar cancer resist a "superinfection" with a second tar cancer, and that a tar tumor will not "take" in a mouse following the extirpation of a previously existing spontaneous tumor. If these observations are correct and also if one is permitted to draw analogies between man and animal, a person with cancer ought to resist the development of a new tumor.[3]

The problem of benign tumors is different in that the condition represents obviously a purely local disease, and for this reason the coexistence in the same person of multiple benign tumors or of a malignant and a benign neoplasm is merely one of casual interest only.

Whether a preëxisting benign tumor provides a suitable soil for the growth of malignant tumor cells and how the latter will behave under the circumstances has been investigated experimentally by Ehrlich as will be told; but it is especially unusual to have the opportunity to study this symbiosis in man.

In the case to be discussed the patient had multiple primary tumors — a leiomyoma of the uterus, and a meningioma in which carcinoma cells were found to be growing.

* Received for publication September 27, 1929.

Report of Case

Clinical History: A white woman, aged 57, entered the Peter Bent Brigham Hospital March 14, 1923 with the complaint of pain in the lumbar region extending down the left leg to the knee, inability to walk and weakness of the right hand. The left breast had been removed twenty-five years ago at the St. Luke's Hospital, New York City, for an unknown cause, and she has been well since that time.

The present illness began with a dull, persistent, bilateral lumbar pain five months before admission. Two weeks later the pain extended down the left anterior thigh and left gluteal region. There also was trouble in walking, night sweats from eight to twelve weeks, and hemoptysis for two weeks, a spoonful in amount.

On physical examination the abdomen was rigid and the left lower abdominal quadrant was definitely tender. Several small nodules were palpated on the left side of the cervix uteri.

The blood pressure was systolic 110, diastolic 60. The blood and urine were normal. The spinal fluid showed no pathological changes.

March 24, 1923. Roentgen-ray examination of the chest disclosed an area of consolidation in the left base. The thorax was slightly asymmetrical, the left side being less expanded than the right. The left diaphragm was higher than the right.

March 31, 1923. The patient was operated upon for a myoma of the uterus. The wound healed slowly owing to a mild infection. The patient coughed up blood.

May 22, 1923. A re-examination of the chest showed a diffuse mottling which resembled miliary tuberculosis throughout both lungs. The left lower base was hazy.

May 31, 1923. The patient died.

Autopsy Report

Anatomical Diagnoses: Left bronchiogenic carcinoma with metastases to bronchial lymph nodes, liver, adrenals, bone and brain. Meningioma, infiltrated by cancer. Leiomyoma of uterus. Absence of left breast removed twenty-five years previously. Acute aortic endocarditis. Infarction of spleen and kidneys, and septicemia.

The body was found to be well developed and well nourished. There was an edema of the ankles and legs.

The pleura on the left side was adherent to the thoracic wall. Numerous tumor nodules could be seen in the intercostal muscles and below the periosteum of the ribs.

The heart showed warty, recent vegetations at the aortic valve.

The left lung weighed 580 gm., the right lung 520 gm. The left lung contained a large tumor mass in the lower lobe and in the posterior axillary line just below the interlobar fissure. The two lobes

were adherent by tumor. In this area the lung was contracted, owing to a newgrowth which caused a puckering of the pleura. On section the tumor mass which was 6 cm. in diameter radiated into the lung in various directions. On dissecting down the bronchus it was found that near the tumor the bronchus was definitely roughened and the newgrowth seemed to be present in its wall. Along the course of the bronchi and peribronchial lymphatics numerous tumor nodules could be seen radiating to the root of the lung where very large lymph nodes were found, the largest of which measured 5 cm. in diameter. This extended down the posterior mediastinum to the diaphragm. The nodes at the hilum of the right lung were quite normal. The right lung was literally speckled with small, white, creamy nodules which varied in size from 1 mm. to 0.5 cm. in diameter.

The liver weighed 1,335 gm. and was normally plastic and friable. However, it contained numerous tumor nodules measuring from 2 mm. to 4 cm. in diameter.

Both suprarenals were enlarged and on section thin suprarenal cortex could be seen surrounding grayish tumor masses.

The first lumbar vertebrae were compressed to 1.5 cm. in width and also apparently contained tumor.

Brain: The brain weighed 1,300 gm. In the left posterior parietal region was a metastatic nodule which arose at the site of the longitudinal sinus. It measured 1.5 cm. in length and seemed to extend into the secondary sinuses. It was definitely adherent to the dura by clot, and its base seemed in places to have infiltrated the dura. When the skull cap was held to the light it showed areas of increased density more marked than one ordinarily sees in a normal skull, and perhaps evidence of infiltration with tumor.

Meningioma: When the calvarium was removed an elevated tumor mass 2 cm. in diameter was found over the right frontal lobe. It was invested by the meninges, being moderately soft and pinkish gray. In gross the appearance of this tumor was that of a meningioma (dural endothelioma).

Microscopic Findings

Lungs: The tumor is made up of a columnar epithelium with an oval, vesicular nucleus and a deeply stained nucleolus. The cells have an adenomatous arrangement and are supported by a fine

stroma. In sections taken from the bronchi the tumor shows invasion of all the coats. The circular muscle layer is markedly thickened. The seromucous glands, however, are intact being surrounded by a thick wall of small, round cells. The tumor invades largely the capillaries and veins.

In the suprarenals the tumor closely resembles that of the lungs, while in the liver the stroma is rather abundant, dense and fibrous.

In the brain the tumor is found as small nodules composed of cells identical by their shape and arrangement with those of the pulmonary newgrowth.

Meningioma: A cross-section of the entire tumor is studied. In areas where the growth is not invaded by cancer it shows the customary histology characteristic of this neoplastic group. There are numerous psammoma bodies. In places invaded by the malignant epithelial cells (Figs. A and B) the cells of the meningeal newgrowth are dissociated, forming a coarse network. The malignant tumor here is insinuated between the meningioma cells, and the individual cells have adhered to the cellular fibers of the meningioma forming bud-like elevations. Cancerous invasion is more conspicuous at the periphery of the meningioma, however the thick fibrous capsule is not invaded by the malignant neoplasm. In areas where the cancer predominates the meningeal tumor shows a good deal of necrosis.

Comment

The early occurrence of widespread metastases in bronchiogenic cancers has been discussed by the present writer elsewhere.[4] The significance of this report lies in the fact that the malignant epithelial tumor diffusely infiltrated the meningioma.

Following the successful experiments with the induction of cancer in laboratory animals, Ehrlich conceived the idea of studying the pathogenesis of the so-called mixed tumors in human beings. For that purpose he [5] and Apolant [6] inoculated animals with mixtures of two or three tumors from different germinal layers, like sarcoma and carcinoma, or chondroma and sarcoma.

When a mixture of a carcinoma and sarcoma was inoculated into animals this led to a tumor known as a *carcinoma sarcomatodes* in which the parenchyma was made up of the malignant epithelial cells, while the stroma was sarcomatous. There occurred then an

amalgamation of the two different neoplastic types which resulted in the formation of a new type of tumor.

By injecting into an animal a carcinoma or a sarcoma with a chondroma no amalgamation occurred and both tumors grew side by side keeping their own properties. By mixing, for instance, a chondroma with a sarcoma it was noticed that the benign tumor contained isolated necrotic areas surrounded by actively growing sarcoma. In some areas the tumors were entirely separated so as to represent two distinct neoplasms.

Heiman [7] in a recent study utilized Ehrlich's procedure to bring forward the claimed infectiveness of epithelial malignant tumors. He inoculated rapidly growing carcinomas and sarcomas of the rat in the center of large spontaneous or transplanted fibromas of the breast of other rats. This resulted in the growth of a malignant tumor with, however, a greatly reduced proliferative activity of the carcinoma. The epithelial malignant tumor continued to remain encysted in the center of the benign tumor while sarcomas grew along the track of the needle infiltrating the fibrous tissue and ultimately escaped into the tissue of the host. The benign newgrowth (according to Heiman) seemed to play an entirely neutral rôle even though highly malignant cells were present in the center. This, then, according to the same author, is further evidence against an organism being responsible for the growth of malignant tumors, for it might be expected that if such an organism were present it would stimulate the benign tumor to become malignant.

It will be seen that the clinical case herein reported imitated very closely the experiments of Ehrlich, of Apolant, and of Heiman. The bronchiogenic carcinoma invaded the meningeal newgrowth without being amalgamated with it, and retained therefore its individual characteristics. It grew actively, squeezing out the "host." The carcinomatous cells had split the solid rows of the meningioma giving the impression that they were utilizing its cellular fibrillae as a means of advance.

A few workers are still uncertain whether malignant conditions induced in animals are akin to those seen in human beings. Ribbert (quoted by Lewin [8]) was of the opinion that experimental and human cancer are probably different diseases and that in the problem of epithelial malignant disease no analogies should be drawn between these two species. This of course was denied in many instances.

The case here described demonstrates once more that even "bizarre" neoplastic conditions induced in laboratory animals by Ehrlich and others are often an exact counterpart of what one sees in conditions encountered in the clinic.

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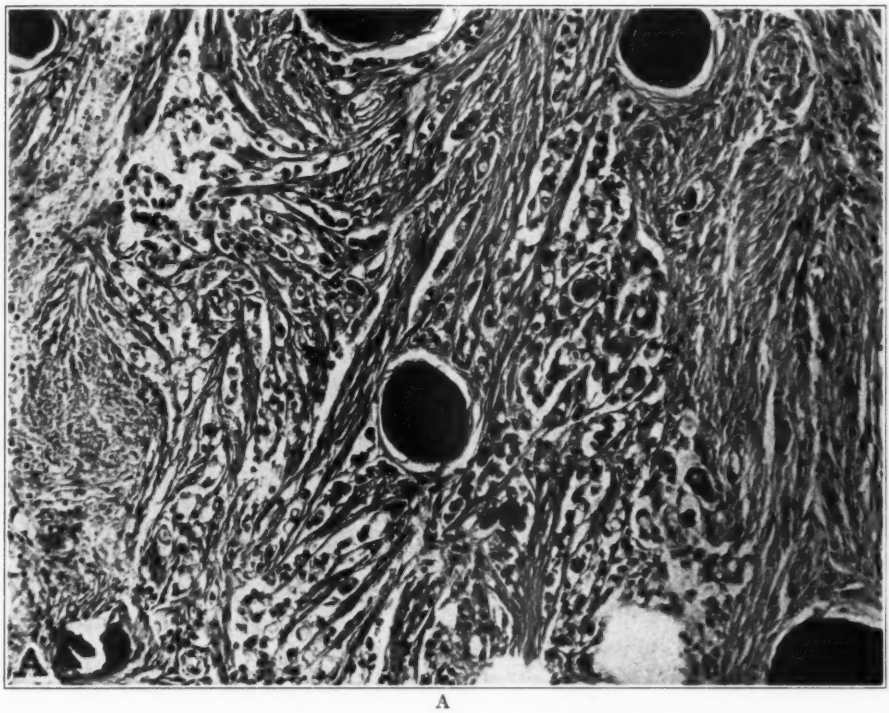
DESCRIPTION OF PLATE

PLATE 16

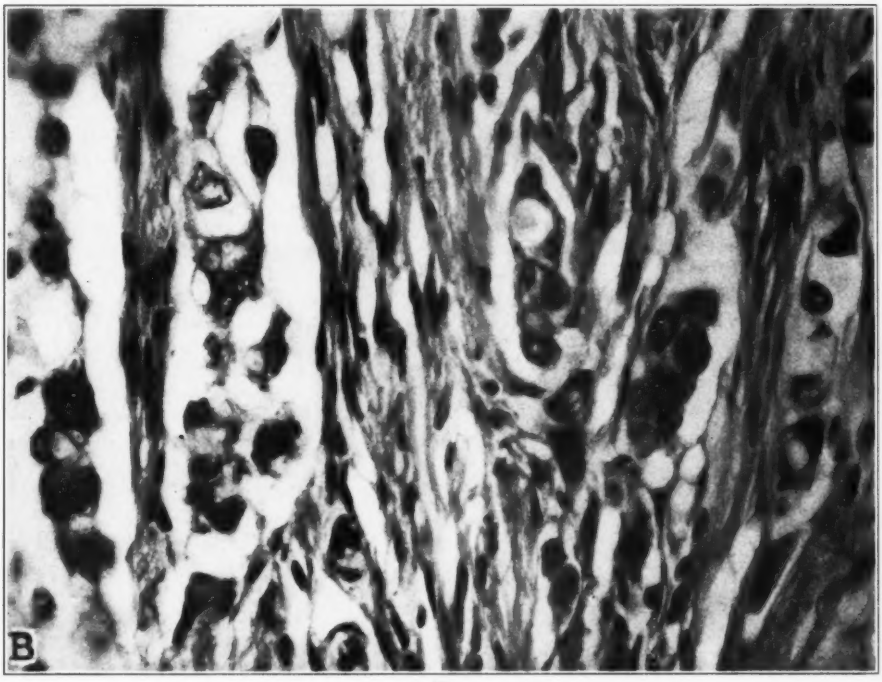
Showing invasion of the meningioma by the cells of a cancer which had originated in the bronchus. Hematoxylin and eosin.

Fig. A × 150.

Fig. B × 635.



A



B

Fried Inoculation of Meningioma by Cancer Cells

STUDIES ON THE SUBMAXILLARY VIRUS OF GUINEA PIGS *

II. The Nuclear Cell, Nucleocytoplasmic and Inclusion-Nuclear Indices of the Affected Cells

Gordon H. Scott and Burchard S. Pruett.

(From the Department of Anatomy, Washington University School of Medicine, St. Louis, Mo.)

In the first paper of this series [1] experiments were reported which showed that the incidence of intranuclear inclusions may be altered at will by experimentally modifying the physiological activity of the submaxillary glands on which the virus acts. Thus, the development of inclusions was found to be greatly aided by stimulation of the gland with pilocarpine, and conversely it was completely inhibited by duct ligation. The latter observation seemed particularly interesting because the operation causes injury and the active formation of young cells — two factors which are believed by Rivers and others to be influential in the promoting of virus action.†

* Received for publication July 1, 1929.

† To explain this discrepancy Rivers has suggested to one of us (G. H. S.) that the virus may be prevented from entering the gland by the operation of duct ligation and that it would therefore be well to inject it directly into the substance of the glands with their ducts ligated. This was done, and as one would expect, inclusions resulted. But the experiment has no bearing upon the interpretation of my results because the conditions in the glands are wholly different. One set of glands is altered merely by duct ligation while the others have superposed upon this change a variety of other kinds of injury caused by the insertion of the needle, the pressure of injection and the local action of a concentrated suspension of very toxic cellular débris as well as of virus. That the conditions are in no wise parallel is further shown by the difference in the cellular response within the glands. After this direct injection of virus into ligated glands there is much congestion, acute inflammation and destruction of cells, and the inclusions appear in several kinds of cells, as contrasted with the mildness of the reaction and the restriction of inclusions to duct cells which are usually observed in unligated glands after subcutaneous inoculation of the virus. The virus reached the unligated glands presumably by the blood stream; that it was prevented from entering the ligated glands by some unknown barrier which unquestionably permits the free access of the blood necessary to the life of the glands, I do not believe. The ligated glands have a rich blood and lymphatic supply and there is no tendency to sequestration by connective tissue formation.

Cellular hypertrophy is often one of the most distinctive results of virus action. In the lymphocystic disease of fish, for example, the volume of the affected cells may be increased as much as a million times. If we are eventually to understand such changes in volume they must be systematically studied by quantitative methods. The submaxillary virus of guinea pigs is perhaps the best to work with, not only because the intranuclear inclusions called forth are larger than those produced by any other known virus, but also on account of the accompanying hypertrophy of the cytoplasm. In addition to these considerations the cells are more or less uniform in shape and are large enough to permit accurate mensurations. In this contribution an analysis of the volumetric changes in cells caused by the submaxillary virus is given.

Material and Technique

The submaxillary glands of four adult guinea pigs spontaneously infected with the virus were selected as material. They were fixed in Zenker's fluid without the usual 5 per cent of acetic acid. Dehydration was carried out slowly and care was taken in all the steps of the technique to avoid undue shrinkage. Serial sections were cut 4 microns in thickness. Some were stained with Giemsa's method, and others with eosin and methylene blue. Because the boundaries of normal duct cells are very indistinct as compared with duct cells modified by the virus, different procedures had to be adopted in gathering data.

Normal Duct Cells: Measurements were made by the methods of Jackson [2] and Covell [3] with but slight modification. Sections passing approximately through the center of the submaxillary glands were prepared. The cut sections of the uninfected secretory ducts were outlined with the aid of a camera lucida on transparent celluloid sheets of standard weight and thickness (Eastman Kodaloid No. 3). The magnification used was approximately 1500. Care was taken to keep the focus of the microscope in a single optical plane while making the tracings. Some 700 nuclei and their surrounding cytoplasm were drawn.

The outlines of the nuclei on the celluloid were cut out with a pair of sharply-pointed scissors, and counted. The areas of celluloid representing the cytoplasm and those representing the nuclei were then weighed separately. Since the magnification and the thickness

of the celluloid remained unchanged, the cut surface relations of the nucleus to the cytoplasm and likewise of the nucleus to the cell could easily be ascertained. The former was determined by dividing the weight of the celluloid representing the nuclei by that representing the cytoplasm, and the latter by dividing the weight of the outlined nuclei by the combined weight of nuclei and cytoplasm. The index of the relation between nucleus and cytoplasm and that of nucleus to cell is obviously one of volumes as well as one of surfaces. As each secretory duct was outlined with its nuclei in a single optical plane it is justifiable to assume that the results are an approximation of the volume relations of the cellular parts.

The celluloid weight of the nuclear areas being known, the average actual nuclear area could be ascertained. This was done by dividing the weight of the celluloid by its weight per square centimeter and further dividing this result by the magnification squared. Assuming that these normal nuclei do not vary greatly in size, the average actual cross-sectional nuclear area may be obtained by dividing this figure by the number of nuclei involved. It will now be shown, on the assumption that the nuclei are spheres of radius R, how the volume of a nucleus can be determined from this area which is denoted by a. It is desired to determine the volume V of a sphere, the average value a of its cross-section being known.

The average area of the cross-section of a sphere is the cross-section of a cylinder which has a height equal to the diameter of the sphere and the same volume as the sphere. Let the cylinder "representing" the sphere have volume V cross-sectional area a, and height h, such that $h = 2R$.

According to the rules of solid geometry the volume of a cylinder is equal to the cross-sectional area multiplied by the height (ah) so that in our case $V = 2aR$. As this is also the volume of the sphere which is given by $\frac{4}{3}\pi R^3$,

$$\text{we have: } 2aR = \frac{4}{3}\pi R^3 \qquad \text{or} \quad a = \frac{2}{3}\pi R^2.$$

Here a is the *known* average cross-sectional area and R is the radius of the sphere whose volume is to be found. From the last equation it follows that:

$$R^2 = \frac{3a}{2\pi} \text{ and } R = \sqrt{\frac{3a}{2\pi}}$$

The volume V can now be determined by eliminating R from the relation $V = \frac{4}{3}\pi R^3$ with the result:

$$V = \frac{4}{3}\pi\left(\sqrt{\frac{3a}{2\pi}}\right)^3 = \sqrt{\frac{6}{\pi}}\ \sqrt{a^3} = 1.382\sqrt{a^3}$$

Having derived the formula $1.382\sqrt{(\text{average area})^3}$, the average nuclear volume is readily calculated by simple arithmetic procedures.* The mean cytoplasmic volume may be computed by multiplying the average nuclear volume by the figure in the nucleocytoplasmic ratio (area of cytoplasm ÷ area of nucleus) representing the cytoplasm to which result the volume of the nucleus is added for an estimation of cell volume.

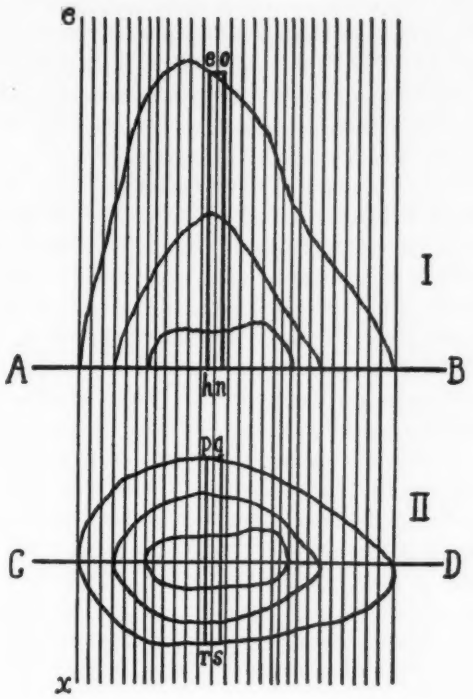
It should be pointed out in connection with this means of determining nuclear and cell volumes that the more nuclei measured, the greater the accuracy of the results. This statement is easily capable of experimental proof by drawing upon ruled paper a circle of known radius and computing the volume of a sphere of the same radius by the usual means ($\frac{4}{3}\pi R^3$). Then determine the areas of sections through the sphere at ten equidistant points along the diameter of the circle and compute the volume of the sphere by the formula $1.382\sqrt{(\text{average area})^3}$. It will be seen that this result is rather far from the true value. If another set of areas is determined at twenty points along the diameter the result will be considerably closer and if forty or fifty readings are made the results approach each other with some exactitude.

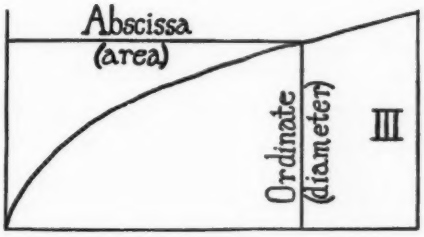
Direct measurements of nuclei of the normal duct cells were made by means of a filar-wheel micrometer at the same magnification. In all, 450 of these measurements were made and used as a check on the calculated radii and on the computed nuclear volumes.

Infected Duct Cells: It is evident that the method used for the normal cell volume is not applicable to infected cells, because the latter do not occur in compact groups but are isolated and surrounded by seemingly normal cells.

After trying out several possible methods the one used by Boyden [4] for determining the volume of the gall bladder was finally adopted. One fundamental assumption is necessary to its use in this connection, namely, that the object be cylindrical or spherical about a central axis. Fortunately infected cells are generally of this shape (see Fig. 1), and care was taken to select those which conformed as nearly as possible to this specification. As an additional precaution wax models of the cells, their nuclei and inclusions, were made in several instances to serve as a check on the cell shape. The method

* The authors are deeply indebted to Doctor Vladimir Rojansky, of the Department of Physics, for helpful criticism and aid in the solution of certain mathematical problems involved in this investigation.





Text-Figure 1

A diagrammatic representation of the method used in determining the volume of the cell, nucleus and inclusion in the infected cells.

finally adopted to determine cell volume was somewhat laborious and is dependent to a certain extent upon the mechanical skill of the observer; but statistical analysis of the data thus secured showed that it was dependable and productive of accurate results.

Camera lucida tracings of the cells, their nuclei and inclusions, were drawn at a magnification of 2060 diameters.

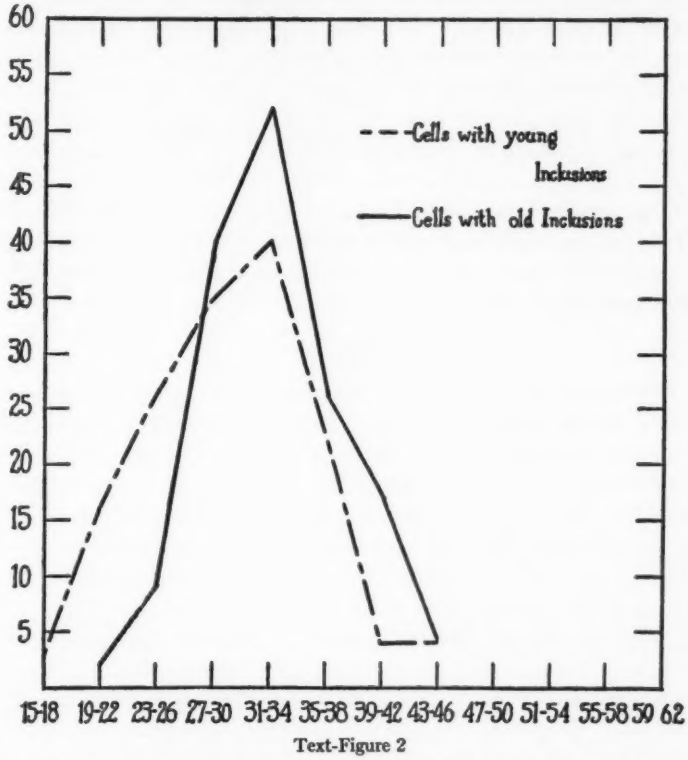
In Text-Figure 1 is a camera lucida tracing of a cell, the nucleus of which contains a mature inclusion body, bisected longitudinally (II). The line *AB* was drawn parallel to the long axis of the cell *CD*. A series of perpendiculars which cut the cell into a number of cylinders of unknown height were erected to the line *AB*. Care was taken to erect the first perpendicular so that it just touched the edge of the cell. The same precaution was observed in passing these lines through the nucleus and the inclusion. With a divider the various diameters of the cell were measured and a distance numerically equal to the area of a circle with the same diameter was laid off from the base line *AB* on each perpendicular. To facilitate the process a graph (Text-Figure 2) was constructed so that the abscissa of any point was equal to the area of a circle with a diameter of that of the ordinate.

As an example let the diameter *pr* be determined with the divider and the distance be made to fall from the base line to the curve at some point. Then let the length of the abscissa from that point on the curve be determined with the divider. This distance may then be laid off from the base *AB* as the line *eh*. When this process has been completed for all segments of the cell, a smooth curve is drawn through all the points. The same procedure is followed through for the nucleus and for the inclusion. It is apparent from elementary calculus that the area enclosed by the line *AB* and by each of the three curves drawn is equal to the volume of the magnified cell and its respective parts. The areas were measured with a planimeter, and, in order to reduce the term obtained to the actual volume, were divided by the magnification cubed (2060^3).

In theory the method is simple. The shape of the cell is considered to be approached by a series of circular discs. Let *pqrs* (II) represent one of these discs as seen from the edge. Then the height *eh* of the rectangle *eohn* (I) is numerically equal to the area of the base of the disc *pqrs* and the width of the rectangle is equal to the height of the disc. Since the volume of any cylinder is equal to the area of its base times its height, then the area of rectangle *eohn* is equal numerically to the volume of the disc. The integrated volume of x number of these discs is therefore approximately numerically equal to the magnified volume of the cell and its parts.

Further considerations of the theory of this method are given by Boyden [4] in describing its application to the human gall bladder in the living subject.

In addition to the quantitative methods for cell study, microchemical procedures were used to obtain information concerning changes other than volumetric. Sections of infected glands from



Text-Figure 2

A point-to-point frequency graph which illustrates the distribution of the observations on the nuclear cell indices of the cells with early and late inclusion bodies. Ordinates: number of observations at given values. Abscissas: nuclear cell indices.

young and old guinea pigs were prepared and stained for the demonstration of mitochondria. The Feulgen reaction for thymonucleic acid was applied to salivary glands according to the directions given by Cowdry.[5] Tests for potassium were made by the Rohdenburg and Geiger [6] modification of the familiar Macallum [7] technique.

Treatment of Data

It was found inadvisable to attempt to treat the ordinary expression of nuclear and cytoplasmic relations, the nucleocytoplasmic ratio, by statistical methods. To avoid this difficulty the following expressions of relation were used:

$$(a)\ \text{Nucleocytoplasmic index} = \frac{\text{Volume of nucleus}}{\text{Volume of cytoplasm}} \times 100,$$

$$(b)\ \text{Nuclear cell index} = \frac{\text{Volume of nucleus}}{\text{Volume of cell}} \times 100,$$

$$(c)\ \text{Inclusion-nuclear index} = \frac{\text{Volume of inclusion}}{\text{Volume of nucleus}} \times 100.$$

The individual cell determinations made on the infected cells were analyzed by simple statistical measurements of variation; namely, averages, standard deviations and coefficients of variability with their probable errors.

Results

Cell Volume: Care was taken to select normal secretory ducts which were of approximately the same size as those in which the inclusions are usually found. The average volume of 747 normal cells measured was found to be 613.2 cubic microns. It is interesting to note that the variation in the four sets of calculations made was of the order of ten. In order to determine the effect of inclusion-laden cells upon the adjacent seemingly normal ones the average volume of 597 unaffected cells in infected ducts was determined. These cells appeared to be, on casual inspection, somewhat compressed and small. But in spite of this appearance their average volume proved to be somewhat over 200 cubic microns greater than that of the normal duct cells. A few nuclear counts were made and it was found that there were in every instance fewer nuclei in such a duct as contrasted with the normal. Nuclear measurements also proved to be greater than in the normal duct. From these observations it is clear that duct cells near others which are infected, though they look normal, react in the usual way to stains and contain abundant mitochondria, are in reality modified volumetrically.

The infected cells were divided into two groups based upon staining reaction and upon the presence of certain cytoplasmic inclusions to

be described in a forthcoming publication. In the first group, classed cells with "early inclusions," were placed those cells in which the cytoplasmic inclusions had not yet appeared and the small nuclear inclusions of which were acidophilic when stained by Giemsa's method. In the second group, designated cells with "late inclusions," the cytoplasm, in contrast contained many inclusions, and the nuclei exhibited large inclusions which were intensely basophilic in reaction when stained by the same method. Such marked characteristics admitted of no shading over from one class into the other in the arrangement of the data nor in the selection of the cells for measurement. Another useful point in classification was that nuclear hypertrophy was marked in the cells with "early inclusions" though the cytoplasm was but little altered. In the selection of material, however, little attention was paid to the cell size because doing so would obviously yield results which did not approximate the truth.

The volume of the cells containing "early inclusions" was not appreciably altered from that of the seemingly normal, but actually

TABLE I

Average Volumes of Cells, Nuclei and Their Inclusions

	Cell volume in cubic microns	Nuclear volume in cubic microns	Inclusion volume in cubic microns	Number of cells
Normal duct cells	613.2	149.1		747
Normal cells in infected ducts	816.0	160.1		597
Cells with early inclusion bodies	872.2	260.8	44.6	150
Cells with late inclusion bodies	2111.9	694.9	210.2	150

slightly enlarged, cells with which they were in intimate association. This group of 150 cells showed an average volume of 872.2 cubic microns. Despite this fact the nucleus had increased by approximately 100 cubic microns. The volume of the cells containing "late inclusions" was larger by more than 300 per cent than that of the normal duct cells, the mean value being 2111.9 cubic microns. The figures dealing with the changes in cellular volumes are given in detail in Table I.

Nuclear Volume: The volume of the nucleus of the normal duct cell, when estimated by the procedure employed, was found to be 149.1 cubic microns. The mean diameter of the nucleus was calculated to be 6.76 microns. The observed diameter of the nucleus averaged 6.55 microns when a control series of 450 nuclei were measured. The mean nuclear volume of the apparently unaltered cells in the infected ducts was computed to be 160.1 cubic microns. This rise in nuclear volume however is so slight that it would be unwise to attach much significance to it.

Nuclear hypertrophy in the cells with "early inclusions" increased the volume of the nucleus to 260.8 cubic microns, but the cytoplasmic enlargement did not keep pace with the nuclear change. The duct cells with "late inclusions" showed an even greater increase in volume for at this stage the average nuclear size reached 694.9 cubic microns (Table I).

Inclusion Volume: The inclusions, found in the cells with beginning infections, when measured showed an average volume of 44.6 cubic microns. The mature inclusions, on the other hand, had increased in size so that they were more than 300 per cent greater in volume than those of the cells with early infections. The volume of the inclusions in these cells was 210.2 cubic microns or almost as large as the entire nucleus in cells with "early inclusions."

Nuclear Cell Index: This expression of the nucleus cell relation in terms of volume was obtained by dividing the volume of the nucleus by the volume of the cell and multiplying the result by 100 so that it could be expressed in percentages. The nuclear cell index for the normal cells was 24.27, which means that the nucleus formed practically one-fourth of the cell volume. The normal cells in the infected ducts showed a definite and significant change in this relation because the nuclear cell index dropped to 20.44. In this instance the nucleus formed one-fifth of the total cell volume. No microscopically visible changes were observed capable of explaining why this alteration in cell and nuclear volume should occur.

Since the infected cells were measured individually it was possible to apply certain mensurations of variability which confirmed the propriety of separating them into two classes. It also provided a means of checking the accuracy of the method applied to their volumetric determinations.

The mean nuclear cell index in the cells with "early inclusion

bodies" was found to be 30.00 with a probable error of ± 0.319, while the same index for cells with "late inclusions" was 33.07 ± 0.263. The difference between these two means is 3.07 ± 0.413. It is important to note that in every test applied, the variability of the cells with "early inclusions" was greater than that of the cells with "matured inclusion bodies." This fact seems to indicate that there is a much more rapid rate of increase in cell and nuclear volume during the early development of the inclusions than later on. Furthermore it seems probable that the cells with "late inclusions" have

TABLE II

Average Nuclear Cell Indices

	Number of cells	Nuclear cell index	Standard deviation	Coefficient of variability
Normal duct cells	747	24.27		
Normal cells in infected ducts	597	20.44		
Cells with early inclusion bodies	150	30.00 ± 0.319*	5.80 ± 0.226	19.33 ± 0.753
Cells with late inclusion bodies	150	33.07 ± 0.263*	4.77 ± 0.186	14.42 ± 0.562

* The difference of these two means is 3.07 ± 0.413 and may therefore be regarded as significant.

reached the peak of their size increase and have become more or less stabilized with regard to volumetric relations.

These figures, with variability determinations, are given in detail in Table II. The scatter of the observations on nuclear cell index is illustrated by the frequency graph given in Text-Figure 2. It is of interest to note that the modal points of the two curves coincide although the means of these two sets of data are significantly different.

Nucleocytoplasmic Index: By the subtraction of nuclear volume from cell volume a figure was obtained which represents the volume of the cytoplasm for a given cell. An index of the proportion of nucleus to cytoplasm was then derived by dividing the volume of the nucleus by that of the cytoplasm and multiplying the result by one hundred. In the normal duct cells the nuclear volume was approximately one-third of that of the cytoplasm. The normal cells in the infected ducts showed, however, a decrease in this relation for the

percentage value was but 24.40, that is to say the nuclear volume was only one-fourth of that of the cytoplasm (Table III).

Here again the infected cells with "early inclusions" exhibited greater variability than that seen in the cells with "late inclusions." The mean value of the nucleocytoplasmic index in the beginning in-

TABLE III

Average Nucleocytoplasmic Indices

	Number of cells	Nucleocytoplasmic index	Standard deviation	Coefficient of variability
Normal duct cells	747	32.13		
Normal cells in infected ducts	507	24.40		
Cells with early inclusion bodies	150	43.79 ± 1.659	12.30 ± 1.173	28.09 ± 2.680
Cells with late inclusion bodies	150	50.16 ± 1.500	11.12 ± 1.061	22.17 ± 2.115

fections proved to be 43.79 ± 1.659; while at the later stage it was 50.16 ± 1.500. Although the difference of the mean values was nearly 7 per cent, the coefficient of variability was so high that the significance of the difference is problematical. The standard deviation was high in both cases.

Evidently, therefore, the relation of the size of the nucleus to the size of the cell is, in infected cells, a more constant proportion than that of the nucleus to the cytoplasm.

TABLE IV

Average Inclusion-Nuclear Indices

	Number of cells	Inclusion nuclear index	Standard deviation	Coefficient of variability
Cells with early inclusion bodies	150	17.07 ± 0.396*	7.19 ± 0.280	41.88 ± 1.631
Cells with late inclusion bodies	150	30.89 ± 0.362*	6.58 ± 0.256	21.16 ± 0.824

* The difference of these two means is 13.82 ± 0.537 and may therefore be regarded as significant.

Inclusion-Nuclear Index: An index of the volumetric relation of the size of the inclusion to that of the nucleus was obtained by dividing the volume of the inclusion by that of the nucleus and multiplying the resulting figure by one hundred. It was found that cells with "early inclusions" showed an index of but 17.07 ± 0.396 while in those with "mature inclusion bodies" the index was 30.89 ± 0.362. The difference here was of unquestionable significance. Again the cells with "early inclusion bodies" proved themselves to be more variable in this relation than their successors (see Table IV).

Discussion

These results are interesting from several points of view. The use of quantitative methods in which definite measurements are treated mathematically eliminates the personal equation in making observations. Though laborious they bring to light cellular changes which otherwise would remain undetected. For example, cells not containing inclusions but situated near others which do, appear to be normal, as judged by their tinctorial properties and mitochondria. Unconscious comparison with the greatly hypertrophied inclusion-laden cells, by the observer, gives the distinct impression that they are smaller than usual (personal equation); but measurements show that on the contrary they are themselves slightly enlarged — a modification which probably might not have been noticed unless quantitative methods had been employed. We do not know what causes this change. The cells are not responding to the virus in the usual way, for this reaction is peculiarly selective involving isolated individual cells and not masses of them. The neighboring cells with inclusions, with which they were compared, themselves represent a response to the virus, probably of several days standing. It does not seem possible that the infection, whatever it may be, passes from the greatly hypertrophied cells to those in the vicinity, for the surrounding cells do not show "early inclusions." All cells with inclusions in an individual duct seem to be in approximately the same stage of development. The chief difference between an infected duct and a non-infected one is that the former contains cells which have been injured as a result of their reaction to the virus. It is possible that these cells while still living or even after death give off a substance, or substances, which cause the slight hypertrophy of the neighboring cells which we have reported.

At the beginning of the reaction to the virus the duct cells are unquestionably alive and engaged in the transport of fluids. It is difficult to explain why some respond and develop intranuclear inclusions while the vast majority do not. The cells affected are not particularly young, for mitotic figures are found only with the greatest difficulty, in fact only one dividing cell was seen in several hundred glands examined. This instance was in a normal secretory duct of a gland from a guinea pig aged three weeks. Another factor which is said to promote cellular response to virus action is mechanical injury, but there is no evidence that such has occurred. From the physiological point of view there are no observations which would indicate that neighboring cells in the ducts may be normally in distinctly different metabolic states. Yet, as we have stated, some cells respond and others do not. It can hardly be a case of the avenue of approach of the virus, which would appear in many instances at least to be by the blood stream, because contiguous cells are evidently supplied approximately equally.

When this barrier against virus action which is effective for most of the duct cells is broken down, the cells apparently invariably succumb. There are no indications that affected cells ever recover. They pass through a series of changes which we have studied quantitatively and qualitatively. In the hypertrophy which takes place the nucleus at first leads the cytoplasm. Thus, in cells with "early inclusions" the volume of the nucleus has increased 75 per cent and that of the cytoplasm only a little over 30 per cent.

The appearance of cytoplasmic inclusions, which have been noted by several workers [8, 9] but have not as yet been studied in detail, marks an alteration in these volumetric relations. From this point onward, the enlargement of the cytoplasm is of much the same order as that of the nucleus. The progressive change is consequently more marked in the nucleocytoplasmic index than in the nuclear cell index. Before the hypertrophy of nucleus and cytoplasm reach their maxima (about 700 and 2100 cubic microns, respectively) the cells are probably dead — a conclusion which seems justified, on cytological grounds, by the following observations:

In cells only moderately enlarged (100–200 per cent) the cytoplasm when stained by Giemsa's method is distinctly basophilic in contrast to its strong affinity for eosin in the normal secretory duct cells, which points to a swing of the reaction toward the acid side. The mitochondria disappear in accordance with

their almost universal behavior in dead cells. The cytoplasmic inclusions, which will be made the subject of a separate paper, persist. Marked changes occur in the nuclei. The chromatin is greatly reduced in amount and is marginated on the the inner surface of the nuclear membrane. Thymonucleic acid is reduced to a minimum. Only the nucleolus remains. The hypertrophied cells contain much more potassium both actually and relatively, a fact which seems to indicate that there is a relaxation in the selective properties of the cell membrane. But this breaking up of the internal mechanism which we are accustomed to associate with life is arrested at a certain point. The nuclear membrane, though modified, invariably persists, and it is always easy to distinguish between nucleus and cytoplasm. Yet the cells do not present the usual features of necrosis. They are in some respects like cysts containing much fluid, though the cell membranes are not thickened nor are they folded, but are kept taut, presumably by internal pressure.

It is interesting that the forces which condition the regular course of cellular hypertrophy seem to operate without break over the period of the supposed death of the cells, for the enlargement continues up to the maximum point with but slight alteration in the nuclear cell index. The cells showing the greatest increase in volume maintain a nuclear cell index within the range of gratuitous variability. The absence of extremely large cells from the series makes it clear that there is an end-point in the size increase not far from the mean volume of 2111 cubic microns.

The final result of the hypertrophy does not appear to be the rupture of the cell and the removal of the débris through phagocytic action (or the mechanical effect of the secretory flow) neither is the cell usually swept away in the stream of secretion. On the contrary the affected cells are remarkably resistant and persist seemingly unmodified for long periods — perhaps even throughout the life of the animal. Leucocytic infiltration is conspicuous by its absence except when the virus is injected directly into the gland. Not only do the cells withstand disintegrating forces *in situ*, but, in contrast to dead epidermal cells containing various types of inclusion bodies, they practically never desquamate except in rare cases in the mucous portion of the gland. This failure to desquamate in the serous part, which is the one almost always affected, is the more surprising because the activity of the adjoining cells, oscillations in hydrostatic values and the passage of fluids are factors which one would expect to favor the removal of cells which like these project far into the ducts.

The reason why the inclusions produced in duct cells by the submaxillary virus are larger than any other specific intranuclear inclu-

sions in mammals — a fact to which we called attention in the introduction — is not necessarily that the virus acts in any way radically divergent from other viruses though its affinities are of course different. It is rather we think a matter of the kind of cell attacked and its relation to the passage of fluids, for we find that on intracerebral inoculation of the same virus into guinea pigs the nuclear inclusions are not of unusual size, nor do the affected cells undergo anything like a corresponding hypertrophy.

On the exact relation of virus to inclusion body we do not wish to commit ourselves. It may be that the virus is present in the inclusion as the findings of Woodruff and Goodpasture [10] would seem to indicate for fowl-pox; but again we have no evidence. Certainly the bond existing between the submaxillary virus and the affected cells would seem to be one of unusual strength. It is indeed unlikely that any other cells of the body are at regular intervals throughout the life of the animal more thoroughly washed with fluids of low salt content. Their sides and distal surfaces are bathed in water, which on entering the lumina of the ducts is thought to decrease the viscosity of the secretion, which itself by constant passage would tend likewise to dislodge any virus adherent to the proximal ends of the cells.

Summary

The volume of infected duct cells is greater by 300 per cent than that of the same type of normal unaffected cells. Nuclear volume is increased more than 400 per cent by the presence of the inclusion body. The nuclear cell index is increased from 24.27 to 33.07 during the course of cellular hypertrophy, while the nucleocytoplasmic index is raised from 32.13 to 50.16. The inclusion-nuclear index is practically doubled by the time the cell has reached its stage of maximum hypertrophy.

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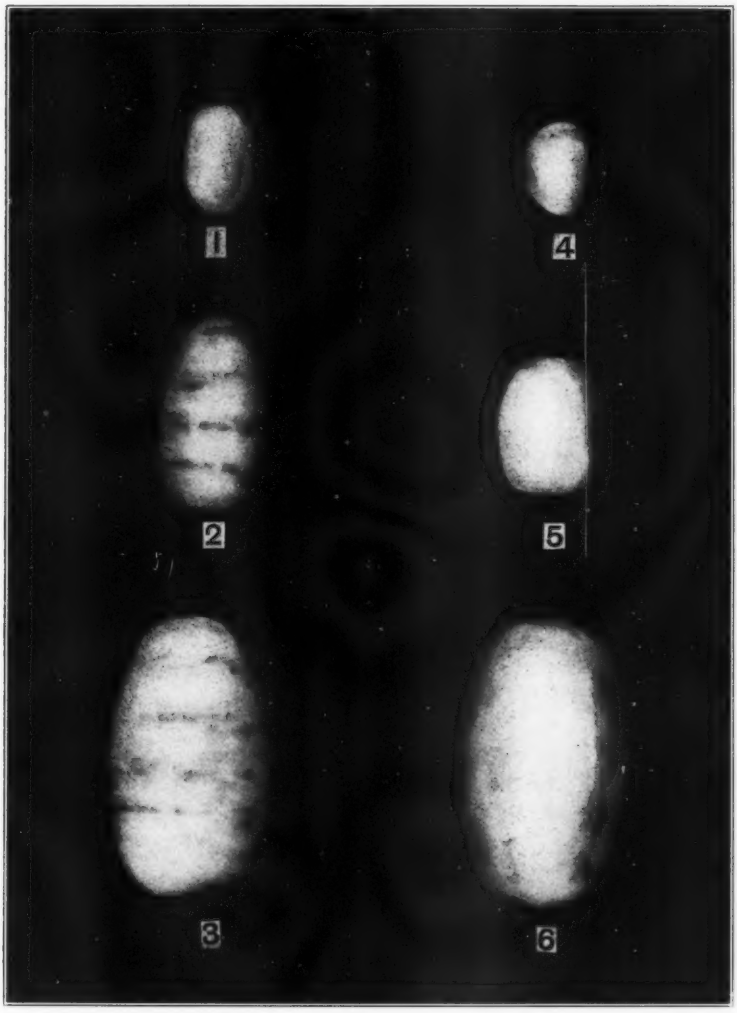
DESCRIPTION OF PLATE

PLATE 17

Figure 1 is a photograph of wax models of two cells with late inclusions, each with its nucleus and inclusion body which illustrate their general shape. Each model was built from camera lucida tracings on wax plates of appropriate thickness for a magnification of 1500^{3}. The sections from which the cells were drawn were cut at four microns and stained by Giemsa's method. Under these circumstances each cell could be identified in from six to eight sections. 1. Intranuclear inclusion of cell number 1. 2. Nucleus of cell number 1. 3. Cell number 1. 4. Intranuclear inclusion of cell number 2. 5. Nucleus of cell number 2. 6. Cell number 2.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CHANGES IN THE THYROID GLAND OF THE GUINEA PIG FOLLOWING A PERIOD OF ADMINISTRATION OF POTASSIUM IODIDE *

JACOB RABINOVITCH

(From the Department of Pathology, Washington University School of Medicine, St. Louis, Mo.)

In previous communications [1, 2] reported from this laboratory it has been shown that the oral administration of potassium iodide to normal guinea pigs produces certain characteristic changes in the histology of the thyroid of these animals. These changes are uniform and consist for the most part in a marked mitotic proliferation of the acinar epithelium as well as in a slight increase in size of the epithelial cells and a slight softening of the colloid; at the same time the number of phagocytes invading the colloid increases greatly. Such changes were produced in the thyroid of guinea pigs that were fed daily with from 0.01 to 0.1 gm. KI for a period of two to three weeks, and in which the thyroids were removed from the animals immediately following the last feeding of the iodide.

It was suggested by Dr. Leo Loeb that it would be of interest to determine the length of time necessary for the thyroid to return to its normal state following the cessation of the administration of KI and to investigate the changes which take place in this organ during this period. The following experiments were therefore carried out.

Fifteen male guinea pigs weighing on the average 400 gm. were fed daily with 0.1 gm. KI for a period of fifteen days. The animals were killed and the thyroid removed for examination at different intervals following the last feeding of the iodide according to the following order:

1. Immediately following the last feeding of iodide — four animals.
2. One day later — one animal.
3. Two days later — one animal.
4. Three days later — three animals.
5. Seven days later — two animals.
6. Fourteen days later — two animals.
7. Twenty-one days later — one animal.
8. Thirty days later — one animal.

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The first four animals in the series which were killed with chloroform immediately following the last feeding of the iodide served as controls. In each case both lobes of the thyroid were cut in serial sections and studied in the manner previously described by us.

TABLE I

Number of Mitoses

Immediately following last KI feeding	One day	Two days	Three days	Seven days	Fourteen days	Twenty-one days	Thirty days
4350	2144	820	200	100	0	0	0
3120	..	..	456	120	0	..	..
1860	..	..	320	..	..	..	..
3250	..	..	..	..	..	..	..
Average 3145	2144	820	325	110	0	0	0

NUMBER OF MITOSES IN THE ENTIRE GLAND AT DIFFERENT PERIODS

In Table I we find the individual as well as the average number of mitoses that have been found in the entire thyroid gland of the different animals at the various periods. It will be noted that the number of mitoses in the glands of animals killed immediately after the last iodide feeding is as usual high, averaging 3145 mitoses: this number is in agreement with figures previously obtained under similar experimental conditions. The animal in which the thyroid was removed one day following the last feeding of the iodide still continues to show a considerable proliferation of the acinar epithelium as indicated by the large number of mitoses (2144), a figure which is only slightly below the average. Two days after cessation of KI administration the number of mitoses in the thyroid gland shows a considerable reduction, as indicated by the figure 820 as compared with 3145 in the controls. Three days after cessation of feeding of KI the number of mitoses is still slightly above the average found in normal animals. At seven days it has reached about the normal count or is slightly below the latter, while at still later periods, namely fourteen, twenty-one, and thirty days, the mitotic activity of the epithelium is reduced to zero. It is evident therefore that the effect of KI in

increasing the rate of cell proliferation in the thyroid epithelium rapidly declines after cessation of KI administration. It cannot be prolonged beyond two or three days following the last administration of this substance, or, in other words, the stimulus to cell division produced as a result of KI feeding is effective only when the latter is fed to the animal, but after cessation of the administration of iodide cell proliferation ceases. There is in addition an indication that following the period of stimulation a phase sets in, in which the mitotic activity of the gland is below that of the normal thyroid.

Microscopic Study: In the control series, *i. e.*, in animals in which the thyroids were removed immediately following the last iodide feeding, the gland showed the characteristic histological changes that have already been described on previous occasions and have been attributed to KI effects. To recapitulate these changes briefly, they consisted in some softening of the colloid and a marked infiltration of the colloid by phagocytic cells. The epithelium was found to be slightly increased in size. In the case of the animal killed one day following the last iodide treatment, the epithelium was still relatively high, but the colloid was somewhat harder and the phagocytes less numerous than in our control cases. The animal examined after two days showed a very similar picture to that found after one day. In the thyroid removed after three days the colloid exhibited a still greater degree of hardness and showed fewer phagocytes; the epithelium, although slightly taller than that in the normal non-treated cases, was lower than that seen in the control KI animals. After seven days no further change in the structure seemed to have taken place, but glands removed from fourteen to thirty days after the last iodide administration resembled microscopically the normal untreated gland in so far as the colloid was more or less solid, containing only few phagocytes, and the epithelium was low in size. This condition was particularly apparent in those animals in which the gland was removed thirty days following the last iodide feeding.

Conclusions

These experiments show that the stimulating effects of KI upon the growth activities of the thyroid gland are manifest approximately only during the time when this substance is being administered to the animal; proliferation begins to decline within a day or

two following its last administration. While the number of mitoses in the thyroid is very high immediately following the last dose of KI, this number approaches the normal count within three to seven days later and seems to be reduced to a point below the normal fourteen to thirty days later. Similarly, the other histological changes in the thyroid are most marked in glands that have been removed immediately after the last iodide feeding, and the picture gradually returns to that of a normal gland as more time is allowed to elapse between the last iodide feeding and the removal of the thyroid gland.

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THE EFFECT OF POTASSIUM IODIDE UPON THE THYROID GLAND OF UNDERFED GUINEA PIGS*

J. RABINOVITCH AND S. H. GRAY

(From the Department of Pathology, Washington University School of Medicine, St. Louis, Mo.)

Loeb [1] has emphasized the importance of changes in weight in his work on compensatory hypertrophy of the thyroid gland. He noticed that compensatory hypertrophy and the effect of potassium iodide upon this process were diminished in animals that had lost weight. Rabinovitch [2] subsequently showed that the mitotic cell proliferation which occurs normally in the thyroid gland of guinea pigs can be completely inhibited by underfeeding. In continuation of these experiments on the thyroid gland, it was thought of value to study the effect of underfeeding upon the thyroid glands of guinea pigs who had been fed potassium iodide. These glands would normally show very marked proliferation [3] of the acinar epithelium, an increase in the size of the epithelium, a softening of the colloid and an increase in phagocytes. Under these conditions, if underfeeding had an inhibitory effect, that effect would be more pronounced. This report is the result of a quantitative study of this subject.

Thirteen male guinea pigs averaging in weight between 400 and 450 gm. were divided into the following three groups: (1) normal guinea pigs fed with KI, three animals; (2) underfed guinea pigs, four animals; and (3) underfed guinea pigs treated with KI, six animals. The experiments were extended over a period of fifteen days during which time the underfed animals lost between 11 and 26 per cent of their original body weight, while the normal animals gained between 10 and 24 per cent. At the end of this period the animals were killed with chloroform and the thyroids removed and studied in a manner already described in previous communications from our laboratory.

1. *Normal Controls Fed with KI:* The gain in weight observed in these animals varied between 10 and 24 per cent during the course of the experiment. We found in these cases a marked proliferative

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activity of the thyroid epithelium, as evidenced by the high number of mitoses. The number of mitoses in the entire gland of the individual animals varied as follows; 2350, 3112, and 3360, with an average of 2940 mitoses. These figures although somewhat lower than figures previously obtained under similar experimental conditions nevertheless indicate a pronounced increase in cell division. Furthermore, the histological changes in the gland were characteristic of KI stimulation and showed a slight increase in the size of the epithelium, a slight softening of the colloid and a marked increase in the number of phagocytes within the colloid.

2. *Underfed Controls:* The four animals constituting this series were insufficiently fed so that they had lost 11 to 22 per cent of their body weight at the end of the experimental period. The number of mitoses in the thyroid of each of these animals was zero, thus showing a complete inhibition of proliferative activity. In addition, the epithelium was lower than normal and the colloid was harder and very scantily infiltrated with phagocytes. The histological changes of the thyroid gland in these cases indicated, therefore, a very much diminished activity: it was very similar to that described in the previous paper by Rabinovitch.

3. *Underfed Guinea Pigs Treated with KI:* In this series the animals lost 17 to 26 per cent of their body weight because of undernutrition; the number of mitoses in individual cases was 60, 0, 50, 0, 0, 0. These figures approach, therefore, very closely those obtained in the underfed animals not treated with potassium iodide. Evidently the under-nourishment and subsequent loss of weight of the guinea pigs is very injurious to the activity of the gland and counterbalances the stimulating effects of the iodide. There does not seem to be, however, a complete parallelism between the degree of loss of weight and the resulting diminution in cell division, for some of the animals that suffered the greatest loss of weight (26 per cent) had comparatively more mitoses (50) than the animals which lost least weight (17 per cent); some of the latter had no mitoses at all. However, the effect of underfeeding was present in all cases and the differences found between different individuals were relatively slight. In addition to the diminution in cell proliferation, the epithelium was found low cuboidal and the colloid mostly solid with only occasional areas of softening, while the phagocytes were diminished in number and observed only in the softened areas.

Discussion

These experiments illustrate that undernutrition plays an important rôle in the activity of the thyroid gland. The manner in which the undernutrition affects the activity of this gland is rather problematic; it may be assumed however, that the diminished food intake deprives the thyroid of a certain amount of its required nutrition, without which it cannot perform its normal function. Under such conditions KI, which normally stimulates the thyroid epithelium toward increased cell division, fails to be effective and the gland assumes a quiescent resting stage showing a low epithelium and solid colloid. Such a histological picture we find to be characteristic of an inactive gland. These experiments add therefore additional proof to our previous conclusion, that the maintenance of body weight of the animal is an important factor in regulating the activities of the thyroid gland. Our experiments furthermore emphasize again the necessity of considering changes in weight taking place in guinea pigs during the experimental period in estimating the effects of various factors on the growth and function of the thyroid gland.

Summary

The underfeeding of guinea pigs to which potassium iodide is being administered results in suppression of cell proliferation of the thyroid gland.

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METASTATIC CARCINOMA IN THE SPLEEN*

Report of a Case

Donald E. Dial, M.D.

(From the Department of Pathology, Yale University School of Medicine, New Haven, Conn.)

Metastasis of carcinoma to the spleen has always been recognized as a rare occurrence. Rokitansky in his text-book of 1855 says that it occurs, and mentions having seen an instance twenty years before. Beginning with Gussenbauer and von Winiwarter [1] in 1876, the incidence of metastasis to the spleen in a large series of cases has been reported from time to time. The literature has been reviewed more or less fully by di Biasi,[2] Chalatow,[3] Geipel,[4] Sappington [5] and Yokohata.[6] Yokohata recently collected the figures from twelve series of cases and found splenic metastases reported in 313 of 17,783 autopsies on carcinoma, an incidence of 1.76 per cent. The figures from individual series vary from those of Paget and of Reichelmann (2.3 per cent and 2.4 per cent respectively) to those of Handley, who found only one splenic metastasis in 422 cases (0.25 per cent). The form of the metastases varies widely from single or multiple macroscopically visible nodules sharply demarcated from the surrounding splenic tissue, or even encapsulated by connective tissue, to diffuse growths infiltrating the spleen and calling forth little or no visible connective tissue response. It is to this latter very unusual form that we wish to call particular attention in this paper. Yokohata believes that the frequency with which microscopic metastases are found varies with the care exercised in looking for them. He studied from five to twenty microscopic sections of the spleen in each of twenty-nine consecutive cases of carcinoma coming to autopsy and found microscopic metastases in ten cases, or 34 per cent. He cites the similar work of Deelman [7] who found microscopic metastases in seven of seventy-five cases in which no gross nodules were seen in the spleen.

By far the most frequent form of the metastases is the isolated nodule, which may or may not be surrounded by a definite fibrous

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capsule. But a search through the literature reveals only twenty cases of a diffuse involvement of the spleen such as reported here. The diagnosis was usually made only microscopically; in most cases the spleen was described grossly as having the appearance of a congested or amyloid spleen: Sappington's case showed "granite-like mottling." These cases are collected here for reference.

Di Biasi found secondary growths in the spleen in fifty of 2422 autopsies on carcinoma (2 per cent); in one of these there was a diffuse involvement with a microscopic picture closely resembling that in the case reported here. Chalatow reports six cases of carcinomatous metastases to the spleen and in all of them one or more sharply outlined nodules were seen. In one the cut surface was strewn with nodules varying in size from that of a pin-head to that of a cherry-stone; in another, besides two large macroscopic metastases, small groups of cancer cells were seen in all parts of the spleen and in the vessels. Geipel reports five cases of diffuse involvement of the spleen, two of which he lists as secondary to "sarcoma" of the bronchus: it seems possible that these two were carcinoma rather than sarcoma and they are therefore included in this series. Two of Kettle's [8] four cases of metastases to the spleen showed a diffuse infiltration with carcinomatous cells. Kraft [9] reports the interesting case of a woman of 49 years who had had pallor and weakness for seven months; she was found to have a lump in the left breast and enlarged axillary glands. A radical amputation of the breast was done and the operative specimen was reported as scirrhous carcinoma. There was no improvement in the symptoms following operation and two months later the spleen, weighing 2000 gm. was removed. The other viscera appeared normal. Microscopically, the spleen was diffusely infiltrated with what was diagnosed as scirrhous carcinoma. The patient improved and was living and well four months after the second operation. Lyter [10] has a somewhat similar case; a carcinomatous breast was removed, following which fifteen X-ray treatments were given; weakness, cachexia and anemia developed and the patient died eight months after the operation. At autopsy the spleen and bone marrow were diffusely involved by metastases. Von Parsch [11] reports seven cases of diffuse carcinomatous metastases in the spleen; unfortunately his report is very brief and omits details. Sappington's case, one of carcinoma of the breast, was in several respects similar to ours. Clinically, electric

treatment and massage had been used; postmortem examination showed diffuse secondary involvement of practically all of the organs, including the spleen. In addition to these cases Marschoff [12] cites Biermer's case with diffuse infiltration of the spleen, the primary source being a carcinoma of the liver.

Report of Case

Clinical History: A colored laundress, aged 65 years, was admitted to the New Haven Hospital with the history and physical findings of congestive heart failure. In addition to this, three years before admission she had noticed a painless lump the size of a hen's egg in her left breast; a doctor diagnosed it as a "wash-woman's tumor" and prescribed some salve, which she continued to use until the time of her admission. She lost 15 pounds in weight during the four years preceding admission. Physical examination revealed an aged, emaciated colored woman, breathing rapidly and with obvious difficulty. The physical findings of interest here were a firm nodular mass in the left breast, adherent to the skin but not to the underlying ribs, and large hard lymph nodes in both axillae, in the right breast and in the supraclavicular fossae. The skin over the left breast was wrinkled but was not ulcerated. There was pitting edema about the left elbow, attributed to pressure of the enlarged glands on the venous circulation. The rest of the physical examination showed the signs of circulatory failure. The red blood cell count was 1,700,000, the hemoglobin 30 per cent. There was no improvement in the patient's condition, but rather a progressive downhill course to death on the tenth day, with signs of congestive heart failure.

Autopsy

The findings on external examination are essentially the same as the clinical findings, except that the edema about the left elbow has disappeared. The mass in the left breast measures 3 by 6 cm.; it begins beneath the nipple and extends obliquely upward and outward, becoming almost continuous with the enlarged, hard confluent nodes in the axilla. This mass cuts with great resistance; it appears grossly to be made up of dense white fibrous tissue, in which there are numerous opaque yellow streaks. The nodes in the axillae and in the right breast are similar in appearance. On opening the abdomen the peritoneal surfaces are found to be studded with small metastatic nodules. Similar nodules are found in the substance of the liver, the kidneys and the adrenals, in the visceral and parietal pleurae and in the pericardium. The spleen weighs 110 gm. and measures 10 by 6.5 by 3.5 cm. It is dark red and firm. The capsule has numerous fibrous tags which bind it to surrounding structures. On section the malpighian corpuscles can be seen and the trabeculae stand out prominently.

Microscopic Examination

Microscopically, the main tumor mass is found to consist largely of dense fibrous tissue, invaded by large cells with large vesicular nuclei, sometimes singly, sometimes in strands and sometimes in groups resembling acini. In the adjacent lymph nodes the relative amount of connective tissue is considerably less, while in the visceral metastases the connective tissue is scant. The metastases are extremely diffuse, groups of cells being found in the connective tissue surrounding bronchioles and in the lymphatics of the lung, in the myocardium, around the central veins of the liver, in the interlobular connective tissue of the pancreas, in the wall of the urinary bladder, and replacing the bone marrow. Attached to the cortex of the spleen are several fibrous tags, in many of which strands of cells similar to those seen in the breast are found. The trabeculae are well marked. The malpighian corpuscles are small and scattered but the cells do not appear unusual. There are a few small round cells in the pulp but most of the tissue is made up of neoplastic cells similar to those found in the breast. They are so diffusely scattered throughout all the sections studied, in small clusters or occasionally in structures resembling alveoli, that the tissue is almost unrecognizable as spleen. An occasional group of such cells is seen in a blood vessel. A Mallory anilin blue stain shows only a very delicate connective tissue framework supporting the neoplastic cells.

The extremely widespread metastases in this case and in that of Sappington's may very well be associated with the ill-advised treatment given, massage in one and the application of a salve, probably also by massage, in the other. The anemia is probably due in part, at least, to involvement of the bone marrow, as in Lyter's case.

Discussion

The pathway by which the spleen is invaded is discussed by di Biasi, Geipel, Sappington, and others. Of course direct extension from a neighboring viscus occurs, but is not to be considered as metastasis. Di Biasi analyzes the pathway in his fifty cases as follows: by direct bloodstream extension, seventeen cases; probably, but not certainly, by the bloodstream, fourteen cases; by lymphatic or direct extension, sixteen cases; uncertain, three cases: that is to say,

in 31, or 62 per cent, of the cases, the splenic metastases were probably due to bloodstream dissemination. In one of Geipel's cases, with the primary seat in the pancreas, the portal vein and several of its tributaries, including the pancreatic and lienal veins, were filled with carcinoma cells; these cells were seen invading the walls of veins in the spleen, but were not to be seen in the capillaries. He therefore believes that the lienal vein was the pathway of extension in this case, and also that retrograde extension through the portal vein from a primary or secondary growth in the liver must be considered in other cases of carcinomatous metastases to the spleen. Von Parsch finds in some of his cases that the large veins are filled with tumor cells while the splenic tissue is unaltered, and offers the same explanation as Geipel. Sappington attributes the splenic metastases in his case to bloodstream dissemination from the secondary growths in the lungs; we believe that to be the pathway in the present case.

The relative immunity of the spleen to successful implantation of metastatic growths has occasioned much discussion and speculation and considerable experimental work. The explanations offered can be divided roughly into two classes; those based on the reaction of the tissue to implanted cells, and those based on mechanical considerations. In the former view, upheld by Chalatow and Kraft, ferments and hormones or the phagocytic cells of the spleen are believed to destroy the invading cells. A mechanical explanation, according to Sappington, can be based on three factors: (1) there are no afferent lymphatics; (2) because of the sharp branching of the splenic artery from the celiac axis the large metastatic cells are carried past and do not enter it; and (3) the rhythmic pulsation of the spleen keeps the tumor cells oscillating and prevents their getting a foot-hold. Of these three the first seems the most reasonable and is in agreement with anatomical facts. As for the second, the sharp branching of the splenic artery does not prevent the spleen from being one of the most frequent sites of infarction, caused by emboli presumably much larger than the tumor emboli. And as for the third, the rate and amplitude of motion in the lungs is much greater than in the spleen, and yet the lungs have frequent metastases. It must be remembered, however, that all the blood of the body passes through the lungs at each cycle, while only a small part passes through the spleen.

The view that the spleen's immunity is to be explained on a mechanical basis does not lend itself readily to experimental proof or disproof. On the other hand, a great deal of work has been done to show that there is, or is not, something inherent in the spleen which makes it unsuitable soil for neoplastic growths. The volume of this work is so great and the results so conflicting and inconclusive that it does not seem worth while to try to cite it in detail.

Tumors which regress either spontaneously or under radiation therapy show an increase in lymphocytes in the surrounding tissue. This observation has led to the opinion that lymphoid tissue exerts a deterrent effect on neoplasms. Attempts to show an increased susceptibility to transplanted carcinoma following reduction of the lymphoid tissue by X-ray, has in some experiments yielded positive, in others negative, results. Similarly lymphocytosis has in some experiments apparently been accompanied by an increased susceptibility; in others no change was observed. Since the spleen is the most convenient source of lymphoid tissue it has been most used for experimental purposes. Many experiments have been reported to show that the growth of transplanted neoplasms is more rapid in splenectomized than in normal animals; that it is retarded in animals simultaneously injected with spleen extract; and that it is still more retarded by extracts of the spleens of animals already affected with cancer. Other experiments by equally qualified investigators, using similar technique and, in some cases the same strain of transplantable tumor, have shown that none of these procedures has any effect whatever on the rate of growth of the tumors. Evidence has been brought forward to show that antibodies are formed in the spleen, lymph nodes, and bone marrow which can be used therapeutically or prophylactically in experimental animals; these antibodies have not been demonstrated by *in vitro* experiments such as the precipitin or complement-fixation reactions. Some investigators have thought that ferments in the spleen destroy the implanted cells. Spleen emulsion has been tried therapeutically on carcinoma in human beings, but without effect. Lieblein [13] incubated spleen extract with carcinoma cells and observed no cytolytic effect on the cancer cells. According to Goldman [14] cancer transplanted directly into the spleen grows as readily as anywhere else in the body. This in itself seems to be a most convincing argument against any specific immunity of the spleen.

In view of these contradictory results it is not surprising that in recent years this line of investigation has been almost entirely given up. The question of the immunity of the spleen in particular and of the body in general is unsettled; undoubtedly the multiplicity of explanations is due to the same cause as the multiplicity of drugs used in the treatment of certain diseases; namely, the fact that none is found very satisfactory.

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